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News

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ECTOPARASITIC NEMATODES OF PLANTS[1]

J. R. Christie

Some people may object to the expression, ectoparasitic nematodes of plants, because, as with most attempts to classify organisms according to behavior, the group to which it refers is by no means clear cut or sharply defined. Using the expression serves one purpose, however—it directs attention to a large and important gap in our knowledge.

A great many soil-inhabiting nematodes feed on the roots and other underground parts of plants largely from the outside without penetrating the tissues, in much the same manner that aphids feed on a leaf. This, of course, is an obvious and long-known fact. I would be doing my colleagues an injustice if I said that the probable economic importance of such species had been overlooked. Various nematologists, Steiner in particular, have called attention to them and emphasized their probable importance. In general, however, these external feeders have not been taken very seriously. They are not discussed in textbooks and only recently have references to their effect on crops begun to appear in the literature.

The fact that they have received so little attention might lead one to suspect that, from a general economic standpoint, these ectoparasites are of relatively minor importance. To make any such assumption would be a serious mistake. Among them are found some of the most devastating of all the plant nematodes. It may not be a gross exaggeration to say that, in the aggregate, these little known external feeders are responsible for crop losses that are as great as the losses caused by all the other and better known plant nematodes combined.

If these ectoparasitic species are so important, why, you may ask, have they received so little attention? There are various reasons. One is that a nematode which does not enter or become attached to the plant may create a situation that is very elusive. When affected roots are examined the examiner may see the injury but he is not likely to find the nematode that caused it—to do this he must examine the surrounding soil. If he examines the soil he will find, probably, the nematode that caused the injury but he will find also a great many others, several of which, so far as appearance is concerned, may seem just as likely to have caused the damage. In such a situation circumstantial evidence is not enough.

Another reason why a situation can be confusing is that we cannot rely too much on symptoms. Species that feed at root tips can produce a type of root growth that is conspicuously abnormal; nevertheless, such symptoms are not necessarily distinctive of nematode injury. Any condition that kills root tips or stops their growth may have a similar effect. Westgate[2] found that toxic concentrations of copper in the soil will cause stubby-root symptoms on celery, corn, and beans, strikingly similar to symptoms caused by the stubby-root nematode. If lateral roots are attacked and their growth stopped just as they are breaking through the cortex, the root system may be sufficiently depleted to retard seriously the growth of the plant without causing abnormalities likely to be recognized as symptoms of disease. The root system will be small with abnormally few fine rootlets, many that would have developed having been nipped in the bud. In cases of this kind crop failures are invariably attributed to some other cause.

Another reason why they are not better known is that ectoparasitic species attain their greatest importance in tropical and subtropical regions. I do not wish to imply that they are of no importance in the North—such obviously is not the case—but merely that as you go southward the overall damage they cause becomes progressively greater. Paradoxically, the soil nematodes of northern regions have been studied more thoroughly than have those of more southern climes and the inhabitants of tropical soils provide an almost unexplored field of research.

As examples of these external feeders I have selected 4 species, namely, the sting nematode, *Belonolaimus gracilis* Steiner 1949; a stubby-root nematode, *Trichodorus* sp.; the awl nematode, *Dolichodorus heterocephalus* Cobb, 1914; and a dagger nematode, *Xiphinema americanum* Cobb, 1913. These, of course, are by no means the only ones of importance but they are 4 of the most important in Florida and some, if not all, are important in other southeastern states.

THE STING NEMATODE.—The sting nematode is a large, slender worm with an exceptionally long stylet. Adults attain a length of about 2½ mm. This species feeds mostly at root tips but also along the sides of succulent roots, devitalizing the tips and causing a moderate amount of necrosis (Fig. 1, D & E). Although it feeds on many different kinds of plants, the list of preferred hosts, when this is known, may not be so large as that of some other species such as the stubby-root nematodes. The sting nematode probably is indigenous to the southeastern United States and pine trees may be among its natural hosts. Crops that may be severely injured include corn, celery, beans, (Fig. 1, B), soybeans, peanuts, millet, and strawberries. This nematode is known to occur from Virginia to Florida but over this region it seems to be confined to certain local areas or to certain fields and apparently to light sandy soils. Although fairly widespread in Florida, it has never been found in

[1] An invitational paper presented at the Symposium on Plant Parasitic Nematodes at the 44th Annual Meeting of The American Phytopathological Society in Ithaca, N. Y., September 8, 1952.

[2] Westgate, P. J. 1952. Preliminary report on copper toxicity and iron chlorosis in old vegetable fields. Proc. Fla. State Hort. Soc. pp. 143-146.

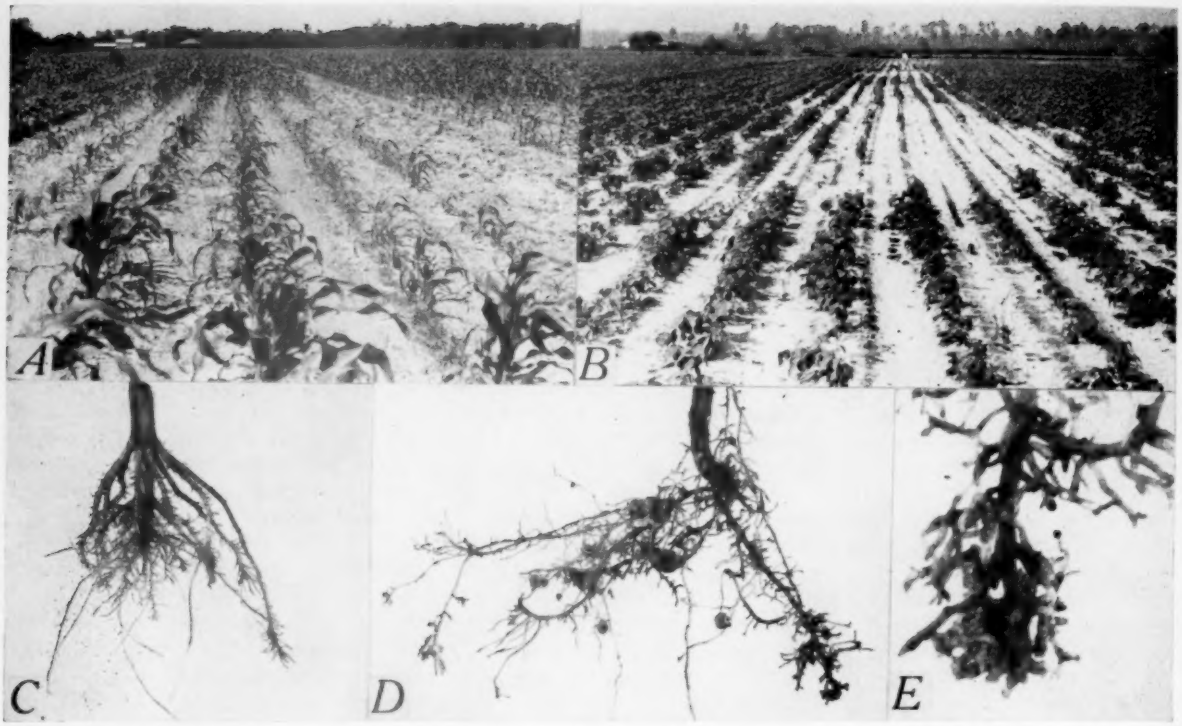

FIG. 1. A) A field of sweet corn near Sanford, Florida, with areas of stunted plants due largely to root injury caused by the stubby-root nematode. B) Field of snap beans near Lake Monroe, Florida, with areas of stunted plants due largely to root injury caused by the sting nematode. C) Root system of a field-grown plant showing severe stubby-root. D) Root system of a bean plant from the field seen in B, showing severe sting nematode injury. E) Part of root system seen in D, enlarged.

muck or marl soils. The sting nematode probably causes Florida growers greater financial losses than does any other plant nematode not excluding the destructive and ever present root-knot nematodes.

STUBBY-ROOT NEMATODES.—Several species of stubby-root nematodes occur in the United States; 2 of these have been found in Florida but only 1 appears to be common and widespread. This one is small and innocuous in appearance with a small, slender, slightly curved stylet. Adults are less than a millimeter long. This nematode feeds almost exclusively at root tips, devitalizing the tips but generally causing very little necrosis (Fig. 1, C). Like the sting nematode, this species probably is a native of the southeast, with grasses and related monocotyledonous plants, possibly including some of the palms, among its indigenous hosts. This species probably attains its greatest importance as a parasite of corn (Fig. 1, A) but beets, celery, cabbage, tomatoes, and chayotes are some of the other crops that may be severely injured. Stubby-root nematodes have been found at various localities in the North. One species is very widespread in Florida and southern Georgia and what is probably this same species has been found in Alabama and South Carolina.

THE AWL NEMATODE.—The awl nematode is very much like the sting nematode in size, in appearance, in feeding habits, and in the injury it causes. Normally an inhibitant of swamps and marshes, it is most likely to occur in wet spots in fields or in land that has been drained. This species has been found in 1 location in Georgia and 2 in Florida, one of these being the Sanford area where it is an important parasite of celery. There is evidence that corn and tomatoes may also be injured. The awl nematode is capable of inflicting very severe injury to the roots of plants and where it occurs it can be very devastating to crops but it does not appear to be sufficiently common and widespread to make it a pest of major importance.

A DAGGER NEMATODE.—The American dagger nematode, *Xiphinema americanum*, is a large slender form with an exceptionally long, powerful stylet. This species has not yet been studied experimentally but observations indicate that it feeds at root tips and along the sides of roots, devitalizing the tips and causing very extensive necrosis. It appears to be the cause of extensive injury to the roots of the laurel oak, *Quercus laurifolia*, a common tree in central Florida and it has been found associated with similar injury to the roots of young pecan trees in Alabama and to the roots of azaleas in Florida. Although it is found occasionally around the roots of corn, oats, and some of the grasses, this dagger nematode probably attains its greatest importance as a parasite of trees and shrubs.

Among the other plant parasites belonging in this group of external feeders are the spiral nematodes (*Helicotylenchus* spp.), the ring nematodes (*Criconemoides* spp., *Criconema* spp., and *Procriconena* spp.), and the pin nematodes (*Paratylenchus* spp.). None of these cause crop losses in Florida that anywhere near equal the losses caused by the sting nematode or the stubby-root nematodes, but none of them can be brushed aside as of little or no importance. Instances are encountered where obviously some of these nematodes are causing serious damage to crops as, for example, a ring nematode in a peanut field in Georgia and a spiral nematode in a planting of amaryllis in Florida.

We have been reading recently articles directing attention to the possibility that, in the not too distant future, the capacity of our agricultural lands to produce food will not keep pace with increasing populations. Regardless of present production in tropical and sub-tropical regions and regardless of what people may think about future possibilities, the potential food producing capacity of these regions is enormous. To what extent can this food producing capacity of the southlands be converted from a potential to an actual one? This is an important question and, should the prognostications of our more pessimistic prophets prove correct, the time may not be far away when it will become a vitally important one. To a large extent the answer depends on our ability to influence conditions in the soil, and soil problems are complex involving many factors of which nematodes are only one, albeit an exceedingly important one, in tropical agriculture.

CENTRAL FLORIDA EXPERIMENT STATION
SANFORD, FLORIDA

DISEASE RESISTANCE BREEDING OF SUGAR BEETS—1918-1952 [1]

George H. Coons [2]

The sugar beet, source plant of nearly one-quarter of the national requirement of sugar, was grown in 1951 on nearly 700,000 acres to supply roots for processing at 67 beet sugar factories. A total of 10,500,000 tons of sugar beet roots were grown, the value of which, in terms of the sugar and molasses obtained from them, was nearly $200,000,000. In addition to these main products, 2 byproducts, beet pulp and beet tops, are highly prized as feed for livestock. They contribute about $100,000,000 more to the returns from this crop.

The sugar beet is grown in 22 states that lie westward from Michigan and Ohio as a central belt across the continent to the Pacific. The distribution of the crop rather closely follows the zone bounded by 67–72° F. isotherms for summer temperature. In 1951, the crop was grown on 27,000 farms. Even with the advance in the past 10 years in mechanization of operations, nearly 100,000 field laborers were required to grow the crop. The average national yield per acre was 15.1 tons, or slightly over 2½ tons of sugar in addition to the byproducts.

In spite of the vigor and adaptability of the sugar beet plant, the industry based upon it has suffered near disaster from certain disease losses, brought about in part by the intensive culture of the crop, depletion of soil fertility (17), and the vast increase of weeds which harbor insect vectors of the curly top virus (24). The diseases which have been most important in the picture are curly top, a virus disease of the west and southwest; Cercospora leaf spot (*Cercospora beticola*), a defoliation disease of the midwest and the more eastern sugar beet growing districts; and black root, a seedling disease complex in which *Aphanomyces cochlioides* is the most important pathogen. This disease is most severe in the humid area. Inroads by these diseases have been greatly reduced by an extensive disease resistance breeding program. This has been accomplished by teamwork[3] of plant pathologists, plant breeders, and agronomists of the sugar beet project of the Division of Sugar Plant Investigations, working in cooperation with State Experiment Stations and the beet sugar companies of the United States.

CURLY TOP.—Probably no plant disease can produce such early and complete crop destruction as curly top of sugar beets. Within a few weeks, affected plants may become so diseased that farmers plow up the fields and abandon sugar beet growing (4). In western United States in curly top epidemic years, as many as 5,000 acres around a factory have been abandoned, the remnant 1,000 or 2,000 acres not so severely diseased being carried through to a meager harvest. Acreages

[1] An invitational paper presented at the Symposium on Disease Resistance at the 44th Annual Meeting of the American Phytopathological Society in Ithaca, New York, September 8, 1952.

[2] Principal Pathologist, Division of Sugar Plant Investigations, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, United States Department of Agriculture.

[3] The breeding of curly top resistant varieties was conducted by Eubanks Carsner, D. A. Pack, F. V. Owen, F. A. Abegg, A. M. Murphy, assisted in the agronomic testing by A. W. Skuderna, C. E. Cormany, J. O. Culbertson, H. A. Elcock, and Charles Price. The breeding of U.S. 15 was by a team consisting of Dewey Stewart, H. A. Elcock, F. G. Larmer, J. C. Overpeck, C. A. Lavis, Charles Price, and the writer. The breeding of leaf spot resistant varieties was by a team consisting of Dewey Stewart, J. O. Gaskill, F. G. Larmer, H. W. Bockstahler, and the writer, assisted in the agronomic testing by J. G. Lill, G. W. Deming, S. B. Nuckols, J. O. Culbertson, C. L. Schneider, and others. The breeding of black root resistance has involved about the same group, with the names of the late J. E. Kotila and of G. J. Hogaboam and R. W. Henderson to be added. Breeding work in California in which nonbolting, rust and downy mildew resistance are stressed, has been conducted by F. V. Owen, J. S. McFarlane, Charles Price, A. M. Murphy, and R. A. Pendleton. The members of the staff of the sugar beet project of the Division of Sugar Plant Investigations have worked in cooperation with the Agricultural Experiment Stations in Michigan, Minnesota, Colorado, Utah, New Mexico, Oregon, and California, as well as with beet sugar companies of the United States.

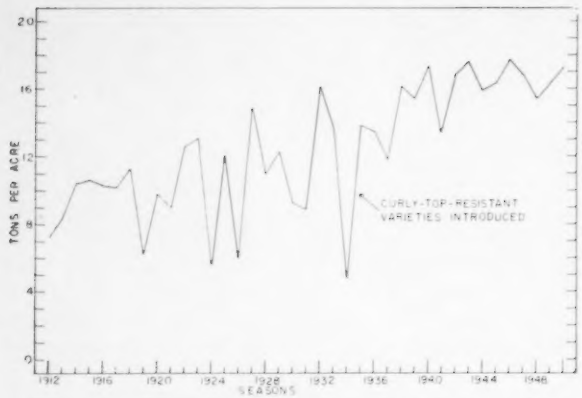

FIG. 1. Average acre-yields of sugar beets in southern Idaho, 1912-1951. (Data from A. M. Murphy).

around several factories had to be pooled so that enough sugar beets would be obtained for a short run for one factory.

Sugar beet yields in southern Idaho, as reported by Murphy (19) and brought up-to-date by figures furnished by him, show the situation before and after introduction of curly top resistant varieties. In 1934, limited quantities of U. S. 1 were available but were in full use in 1935. The effects of curly top on sugar beet yields in southern Idaho were paralleled in western Colorado, Utah, Idaho, Washington, Nevada, and California. Until curly top resistant varieties were introduced, the years of curly top outbreak were characterized by a short crop and heavy loss of acreage.

To meet the depressed situation of the western sugar beet industry, curly top resistant varieties were developed by making mass selections of the sugar beets surviving in the fields that were severely injured by curly top. When curly top attacked early, every plant in the field became affected and all except a few were curled and stunted. The complete and nearly uniform exposure, coupled with the severe reaction of the sugar beet plants, guided the breeder in selecting the few plants that seemed less severely injured, and presumptively were resistant. Mass selections of this type were made by Carsner, Pack, and their colleagues from a number of commercial varieties. These were pooled with roots obtained from similar selections made earlier by commercial companies to produce the first curly top resistant variety, U. S. 1 (5). Although only moderately resistant—about ¼ of the plants in the population were resistant—in comparison with the almost complete failure of sugar beet plants grown from European seed, U. S. 1 was outstanding. It was quickly followed by U. S. 33 and U. S. 34 (1), U. S. 12 (23), and now by U. S. 22 (6, 20). Each of these varieties was more resistant than the stock from which it was selected—that is, the populations successively contained higher and higher proportions of resistant plants. New improved varieties were bred and quickly released to the farmers. For the intermountain area where curly top exposure is most severe and for spring plantings in California, the curly top resistant varieties obtained in this breeding program have, since 1935, constituted almost exclusively the planting stock from which crops are grown.

The curly top resistant varieties have removed the threat of crop failure from the western sugar beet industry. In epidemic years, the toll levied by this virus disease is still considerable, but as a disease of sugar beets, curly top is no longer of primary importance. The resistant varieties have made a highly important contribution to western agriculture by saving for irrigated agriculture its keystone crop, the sugar beet. The success of pathological and breeding techniques in controlling this virus disease has helped focus attention on disease resistance breeding for other serious virus diseases, especially with other economic crops seriously affected by curly top.

SUGAR BEET SEED ENTERPRISE.—To bring the new curly top resistant variety to farmers in adequate supply and without delay, as well as to provide for the future increase of improved varieties, it was necessary to establish a domestic sugar beet seed enterprise. Up to 1935 the sugar beet industry almost exclusively depended on seed imported from Europe. It was shown, in cooperative work with the New Mexico Agricultural Experiment Station, that sugar beet seed could be efficiently and economically produced by planting seed in early fall in drill rows about 20 in. apart, allowing the plants to stand unthinned in the field over winter (21). The field overwintered plants produced their seed in early summer of the next year, the seed crop being harvested by machine. This is in sharp contrast with the conventional European method in which stecklings are planted in late spring, grown in 1 season, then lifted, topped, and stored over winter in cellars or pits, and then replanted the ensuing spring as spaced field plantings.

When the seed of the curly top resistant variety U. S. 1 was first released to beet sugar companies, it was required that the reproduction of seed to supply their growers—both the primary and subsequent increases—be made in United States, subject to control by the Division of Sugar Plant Investigations. To obtain the foundation supplies of seed, beet sugar companies operating in western United States accepted the government requirement and contracted with farmers in New Mexico, Utah, and California to increase the sugar beet seed. The first crops were highly successful.

The domestic growing of seed of adapted varieties quickly became an efficient enterprise. Now all the sugar beet seed planted in the United States to grow the commercial crop for sugar is grown in the United States. By 1938, 6,761,000 pounds of the curly top resistant varieties were grown on about 3,000 acres. In 1941, a strong increase in production took place when European sources of seed were shut off by World War II. A total of 10,235 acres of seed of improved varieties were planted, on which 18,088,200 pounds of seed were produced, enough to meet national requirements and to provide seed for our allies (Table 1).

LEAF SPOT (CERCOSPORA BETICOLA SACC.).—The sugar beet industry east of the Rocky Mountains had

TABLE 1.—*Sugar Beet Seed Production in United States—1937-1951.*

Period	Total Production	U. S. Curly Top Resistant Varieties	U. S. Leaf Spot Resistant Varieties	Stock Seed of Improved Varieties	Improved Varieties Bred by Beet Sugar Companies
	(100-lb. bags)	(100-lb. bags)	(100-lb. bags)	(100-lb. bags)	
1937-1941	130,238	61,261	12,658	895	55,284
1942-1946	142,592	37,999	20,464	1,037	83,565
1947-1951	151,948	49,768	26,314	1,112	72,754

in Cercospora leaf spot a counterpart to curly top of the west. This fungus disease often occurred as an epidemic over wide areas, blighting foliage and reducing yield and quality. Although less spectacular than curly top, its effects were no less damaging, since it could make the crop unprofitable to both farmer and factory. Epidemic years were those in which the first half of the season had frequent rains or periods of high humidity. In the more eastern districts, abundant rainfall in the late spring or early summer brought about an outbreak of leaf spot. Thus, conditions that should have been conducive to highest yields so favored the pathogen that the sugar beet crops were disappointing. In such seasons the foliage blighted several times, each new growth of leaves being at the expense of root growth and stored sugar. In the period 1915–1940, blight years recurred frequently; in some districts sugar beet growing was abandoned.

Mass selection, so successful with curly top, did not work with Cercospora leaf spot. Outstanding plants were not found in heavily blighted fields. Furthermore, as sugar beet leaves mature they become more subject to attack by the fungus, making recognition of potentially valuable individuals almost impossible. When the disease resistance breeding project began in 1925, there was available at Fort Collins, Colorado—as a result of years of work by the late W. W. Tracy—a large array of sugar beet inbred strains separated out of European brands of sugar beet. Tracy had been at work nearly 10 years sorting the sugar beet into its component morphological types. When about 200 of the Tracy inbred strains were grown in 1925 at Rocky Ford, Colorado, under conditions of severe leaf spot, a few strains were found to be outstanding because of their milder reaction to the disease. All other strains had blighted severely but these resistant ones remained relatively green. The strains recorded as outstanding at Rocky Ford gave similar performance at Fort Collins where leaf spot was also severe. From this experience it was immediately evident that to obtain leaf spot resistant varieties, selecting from the strains showing resistance and continued inbreeding would be required until factors for resistance were concentrated and stabilized within a strain. Such work was begun by the leaf spot resistance breeding team, using the Tracy and other strains as starting points. The inbreds rapidly became stabilized for the characters governing resistance, but there was definite loss of vigor. Hybrids between 2 relatively nonvigorous inbreds were found in 1932 to show strong heterosis (29). Breeding for leaf spot resistance resolved itself into producing as many distinct leaf spot resistant inbreds as possible, then finding, by a series of matings, those pairs that would give a strong heterosis response, satisfactory leaf spot resistance being maintained.

The first leaf spot resistant variety introduced in 1937 (U. S. 217) was made from 5 inbreds, all with better-than-average leaf spot resistance (7). Seed stocks of the 5 were pooled, and plants were grown in the seed field from the seed mixture so that all types could intercross. The variety proved to be very resistant to leaf spot, high in sucrose, but somewhat low in root yield. It was soon replaced by U. S. 200 × 215 (12), which was obtained by pooling the seed of Inbred U. S. 200 and Inbred U. S. 215 and planting the mixture in the seed field. Since the sugar beet has perfect flowers, and among inbreds there is more or less tendency toward self-pollination, a variety produced from a planting stock made by pooling of seed consists not only of the hybrid but of sibs of parent varieties as well. The proportions of these classes cannot be predicted and may vary from field to field. It was hoped that in U. S. 200 × 215 about 40 per cent of the progeny would be hybrid. Heterosis shown by a hybrid portion is more or less nullified by the lower yields of inbreds in a progeny. Inbred U. S. 215 was noteworthy among all available strains because it was highly productive, about equaling European commercial varieties in root size. Its sibs probably did not bring about depression of yields.

U. S. 200 × 215 was ready for introduction in 1939 when the European war cut off supplies of foreign-grown sugar beet seed and, as already noted, forced the American beet sugar industry to move 100 per cent into domestic seed production. Seed supplies of the inbreds, U. S. 200 and U. S. 215, were available from Federal sources to furnish the planting stock from which commercial seed for the affected districts of the humid area could be produced, thereby averting a sugar beet seed crisis such as that of 1914–18. The variety supplied was not a mere stopgap but an improved, disease resistant variety about 5 per cent more productive than the European varieties it replaced.

By continued breeding, new leaf spot resistant varieties have been introduced, notably U. S. 215 × 216 (13), which, in its current phase, is designated as the third release of the hybrid, i.e. U. S. 215 × 216/3. This variety without leaf spot is equivalent in sugar production to the European varieties previously grown. Under conditions of leaf spot exposure it is significantly superior to susceptible European types in sugar production. The leaf spot resistant varieties have shown an-

other advantage that comes from their ability to retain a full foliage bouquet in spite of leaf spot. In mechanical harvesting, if leaf spot has blighted down the foliage, effective functioning of certain types of harvesters is prevented. All acreage in Michigan, Ohio, and Wisconsin is planted with U. S. leaf spot resistant varieties (Table 1).

RESISTANCE TO DOWNY MILDEW, RUST, AND TO BOLTING COMBINED WITH CURLY TOP RESISTANCE.—The achievement in breeding curly top resistant varieties only partially met the needs of California because varieties such as U. S. 33, U. S. 12, and U. S. 22, if planted in late fall or winter, produced a high percentage of seed stalks. Hence, the new curly top resistant varieties could be used only for that portion of the crop that was planted in late March or early April. In sharp contrast to the easy bolting characteristics of these varieties was the nonbolting character exhibited by U. S. 15. This variety had been selected in 1927 in cooperation with the New Mexico Agricultural Experiment Station. Plants, highly resistant to curly top, were selected from a high sucrose European variety (R&G Pioneer) that were able to grow in spite of a severe epidemic of curly top. Of 10 roots selected, only 3 produced seed. The progeny of this selection was immediately noted as showing curly top resistance and seed increases were started. The variety was about to be dropped because of its extreme susceptibility to Cercospora leaf spot in Fort Collins screening tests when its tendency toward low bolting was discovered in tests at Davis, California, where a number of varieties were being tested to find nonbolting types. The variety also was found to be very resistant to downy mildew (*Peronospora schachtii*) and to rust (*Uromyces betae*), both serious diseases in California coastal districts (14).

U. S. 15, therefore, was a remarkable sugar beet in that it combined with its curly top resistance (found to be moderate in degree) the tendency not to produce seed when planted in fall or winter when normally these plantings would induce seed stalk production in sugar beet varieties, and in its resistance to 2 serious fungus diseases of sugar beet. U. S. 15 was particularly suitable for winter plantings in central and southern California. Introduced into commercial use in 1938, the variety until recently has been the chief one grown in all winter plantings in California.

The most noteworthy achievement of U. S. 15 was to open for sugar beet culture the Imperial Valley, a contribution which culminated in the erection in 1948 of a 7 million dollar beet sugar factory at Carlton, California. To produce the sugar beet there it is necessary to plant in September or October and harvest in May or June. With ordinary sugar beet varieties such a planting schedule cannot be followed, because the onset of cold weather shortly after the plants emerge and the continued cold weather of November and December cause so many plants to bolt as to reduce crop yields greatly and make harvest operations very difficult. Sometimes there is a light exposure to curly top in the Imperial Valley; hence, for this Valley and for winter plantings in southern and central California, U. S. 15 proved to be remarkably well adapted.

U. S. 56 AND U. S. 75.—After a 10-year period of dominance, U. S. 15 was surpassed by another nonbolting variety, U. S. 56, bred in California by Price and Owen (25). This variety has proved superior to U. S. 15 whenever curly top was a factor. It does not bolt in winter plantings. As fast as seed stocks become available, U. S. 15 is being replaced by U. S. 56.

U. S. 56, in turn, is about to be replaced—after 2 or 3 years of utilization by farmers—with a new nonbolting variety, U. S. 75, which manifests very high curly top resistance and high resistance to downy mildew (18). Its curly top resistance is extremely marked, about equal to that of U. S. 22/3, at present the chief variety of intermountain areas where curly top is serious.

U. S. 15, U. S. 56, and U. S. 75 are stressed because they illustrate the possibility of combining within 1 variety the genes for resistance to many diseases as well as the genes for desirable physiological and morphological characters. Starting with a base of curly top resistance, it is possible to maintain this resistance and select in still other directions, combining new characters in the variety that has the primary and indispensable quality of resistance to curly top.

BLACK ROOT RESISTANCE.—For years, in Michigan, Ohio, and other States, the poor stands brought about by seedling diseases have been, probably more than any other cause, a limiting factor in production. The damping-off diseases, commonly lumped under the term black root, are responsible for the low average yields in the years when rainfall is normal or above. Analysis of the disease situation over a number of years determined that in the damping-off complex, *Aphanomyces cochlioides* was most important in causing crop damage. *Pythium* spp., *Phoma betae*, and *Rhizoctonia* spp., which bring about an acute seedling disease and reduce stands, are limited in their effects but are amenable to control by seed treatment. The chief black root damage has come from *A. cochlioides*, which produces a chronic disease whereby the small feeding roots are killed about as fast as they are formed, so that the young sugar beet may fail to make effective contact with the soil (11).

The attempt to control the chronic type of black root by breeding was based on the observation (8) made in 1941 and 1942 that certain varieties in the leaf spot resistance tests were so badly injured by black root that they could readily be identified by their reduced stands and by weak, stunted growth. Conversely, Inbred U. S. 216 was outstanding in all tests, showing very marked resistance to black root. Here was evidence that within the sugar beet complex, factors for susceptibility and for resistance exist.

The program for development of black root resistance has been carried on in fields of Minnesota and Michigan that had previous histories of crop failure. Since leaf spot resistance is necessary for any variety to succeed in the humid area, inbreds and synthetic varieties from this resistance project have been utilized

as sources for the black root resistance selections. The work started in 1944 at Minnesota with some mass selections by J. O. Culbertson, chiefly from U. S. varieties being grown in tests for leaf spot resistance. Henderson and Bockstahler (16) and Bockstahler and Reece (2) continued the mass selections in 1945 and 1946. The research was extended in 1947 to include Michigan, breeding stocks being interchanged between the stations (15).

As a result of the continued mass selections and, more recently, use of the polycross breeding method, several varieties are now available that retain the leaf spot resistance of the parental strains—probably adequate for all ordinary exposures—and in addition show very definite resistance to black root as caused by *A. cochlioides*. The first release, U. S. 1177, in 3 tests in 1951 in which black root was a factor, produced an average of 600 lbs. more sugar per acre than a susceptible commercial variety. Three varieties that were essentially reselections from U. S. 1177 for black root resistance exceeded it by the significant amount of 400 lbs. of sugar per acre, indicating that very definite improvement had been achieved by the second selection. These promising results indicate that by broad-base mass selections the factors for black root resistance can be incorporated in the leaf spot resistant varieties.

The existence of biotypes of *A. cochlioides* is not now known. In general, inbreds resistant in Minnesota are resistant in tests at both East Lansing and Blissfield, Michigan (3). However, certain outstanding selections in Minnesota tests fail in Michigan, or vice versa. U. S. 216 has shown resistance to black root in all areas where it has been grown; the superior varieties such as U. S. 1177 and the reselections from it are highly resistant to black root in Maryland (Beltsville), Michigan, Ohio, and Minnesota tests. It would appear that in the mass-selected varieties there is achieved an overall inclusion of resistant types and these, with the advantage that comes from heterosis, aid the variety to succeed.

LEAF SPOT-CURLY TOP RESISTANT VARIETIES.—As a further example of possibilities of combining the characters for disease resistance, results obtained by Dewey Stewart in crossing strains highly resistant to curly top with inbreds highly resistant to leaf spot may be cited. There is a need in certain sections of California and other States for a variety with such qualities, since both curly top and leaf spot are known to cause serious damage. In this research, the leaf spot resistant variety has commonly been the recurrent parent in a series of backcrosses. The backcross generation is grown at State College, New Mexico, and subjected to severe curly top exposures so that resistant plants of the population could be selected. Then the material is reselected for leaf spot resistance at Beltsville, Maryland, where severe leaf spot exposure is induced by artificial irrigations. A resultant variety, S.P. 50104-0, is now almost as curly top resistant as U. S. 22 and is adequate in leaf spot resistance to meet the needs of most areas. The curly top-leaf spot resistant varieties are still under trial and may find their place in sugar beet districts where both diseases are serious.

NEW BREEDING TECHNIQUES.—In the sugar beet breeding program, the production of stabilized strains having as their prime quality resistance to either leaf spot or curly top, and secondarily, possessing resistance to other diseases, is becoming increasingly important. In many cases it is necessary to produce a hybrid variety, and here the male sterility factor as discovered by Owen (22) is a valuable tool. By a series of backcrosses, it is possible to produce male sterile equivalents of a sugar beet strain, be it leaf spot resistant, black root resistant, or whatever may be chosen. Then this variety can be pollinized by a desired male fertile strain to produce the commercial hybrid variety.

In the production of U. S. 215 × 216 (9), and in some of the newer leaf spot resistant varieties shortly to be introduced, 1 variety is of male sterile type and this is mixed at about a 3:1 ratio with the variety that is to serve as the pollinator. The mixture is then planted in the seed field. Even if the male sterility character exhibits itself only to the extent of 75 per cent in 1 strain of the mixture, there will be a very strong tendency for a high percentage of the seed of this strain to be hybrid. Newer breeding stocks of the leaf spot resistant sorts are available in which about 90 per cent of the population is male sterile, and their utilization should greatly step up the proportion of hybrids in the commercial seed that is produced. It is probable that when completely male sterile varieties are available, then the pollinator can be planted at the border of the field and as central strips, and not be harvested at all, the 100 per cent hybrid seed being taken from the parts of the field planted with the male sterile parent.

Recently, V. F. Savitsky (27) discovered an inbred that produces its fructification as a single true seed within the pericarp—a monogerm instead of multigerm seed ball. Savitsky has proved that single-germness is governed by a recessive Mendelian character so that by hybridization and by selection in the F_2 generation monogerm individuals carrying the desired characters of 1 parent may be obtained. Obviously backcrossing is a faster way to incorporate monogermness in a given strain. A leaf spot resistant inbred was crossed with the monogerm inbred. Monogerm plants were selected by Stewart in the F_2 generation; the F_3 progenies from the selections have now been tested for leaf spot resistance and other characters. Within the segregates, progenies with very high leaf spot resistance have occurred, whereas others are highly susceptible. Many of the new monogerm types are very vigorous and otherwise attractive. These are the first steps in developing monogerm leaf spot resistant types (28). A similar program is going on with the curly top resistant sorts.

VIRUS YELLOWS.—The sugar beet in United States (10) is now confronted with what may be a new hazard—virus yellows, long recognized as the most serious disease affecting the sugar beet crop of Europe. The disease occurs in Michigan, Colorado, Utah, Oregon,

Washington, and California. So far, in all States but Oregon, Washington, and California, the disease seems to be localized around points where beets have overwintered and thus perpetuated the inoculum. In California, and probably elsewhere on the Pacific Coast, both the disease and the aphid vectors seem to have no off-season. The disease has been known at least since 1945 in the Salinas Valley and received the local name of "Salinas yellows." Sugar beet fields become yellow early in season and it is likely that spinach, which is also affected by this disease, is a common source of both virus and aphid. The effects of virus yellows on the crop in United States have yet to be appraised.

The attempts made by European investigators to find factors for yellows resistance in sugar beet and in wild species of *Beta*, are disappointing. Selections of apparently resistant individuals were made over a number of generations but no increased resistance was demonstrated in progeny tests. In such selections the chance is always great that disease escapes may be included within the group. These serve to prevent definite advance from being registered. Promising results were noted in tests at Bergen-op-Zoom, conducted by Rietberg (26) and his associates. Certain mass selections, as well as inbred varieties, had been inoculated uniformly with the yellows virus. When the experiments were viewed by the writer in 1951, some of the inbred varieties—including certain U. S. 216 inbreds—stood out from the general population by having fewer yellow plants in the plots. The variety showing the fewest yellowed plants had the outer leaves of other plants thickened slightly and up-curled. In their present status, the varieties are considered by the Dutch investigators as "tolerant." The problem is complicated because virus yellows in Europe appears to consist of a number of strains, some of which are more virulent than others. A test in England was seen by the writer in which all varieties, in spite of a history of selection against virus yellows, were uniformly and seriously injured by a necrotic strain with which all plants had been inoculated.

In June 1952, very pronounced differences in varietal reactions were noted at Salinas, California, where incidence of virus yellows infection approached almost 100 per cent. In a variety test at Salem, Oregon—where many inbred strains and varieties were being brought to seed—certain inbreds noted as not showing serious virus yellows in California were essentially free from the disease. Four strains obtained by J. S. McFarlane at the U. S. Sugar Plant Field Laboratory at Salinas, California—NB6, SL 0502, SL 8255, and SL 0504—by inbreeding curly top resistant strains to obtain the nonbolting character, showed very low percentage of plants with virus yellows in California and still lower in the Salem, Oregon, test. A leaf spot resistant inbred, U. S. 201-B, also was noted as essentially free from virus yellows. Another inbred, U. S. 216, showed 25 per cent of its plants affected with virus yellows, as contrasted with U. S. 225 with 100 per cent infection. In this test, many commercial varieties and many inbreds, including the monogerm variety SL 101, showed 75 to 100 per cent infection with virus yellows. Two dates of planting were used in the test, and there was excellent concordance between the 2 plantings.

Observations made in 1952 in Colorado on inbreds developed by G. W. Deming revealed that among strains some were nearly 100 per cent affected with virus yellows, others were infected in a lower percentage, whereas a few were almost free from the disease. The inbreds were being grown as single-row plots at Fort Collins under fairly severe exposure to virus yellows. In a seed increase plot at Virginia Dale with extremely severe exposure, similar differences in virus yellows reaction among inbreds were noted. Possibilities, therefore, seem to be opened for combating this disease by breeding for resistance.

Discussion.—Disease resistance breeding with sugar beets has been successful in producing varieties that meet the needs of the industry far better than those imported from Europe. Less than a score of years ago, European seed was used almost exclusively by farmers to grow the sugar beet crop. Now no sugar beet seed is imported.

Several methods have been employed in the disease resistance program. The plant pathologist has cooperated with the breeder to make sure that disease exposures were adequate. Mass selection has been used wherever possible. Inbreeding and hybridization techniques have been followed when such was required, and reselection within desirable strains has been employed under appropriate conditions of disease exposure to add new characters. In breeding for black root resistance, the polycross method to locate outstanding individuals that would contribute black root resistance has been followed. After meeting the major disease threat, attention could be turned to the improvements that better adapt the beet to special environments.

Much of the material is now approaching a state that will permit closer attention to the factors contributing resistance and the nature of their inheritance. With curly top, the factors governing resistance are dominant, and it would appear that an allelomorphic series is concerned. There is evidence that the genes governing curly top resistance are on the same chromosome that carries the factor for red hypocotyl color. With leaf spot resistance, several factors seem concerned, and crosses between highly resistant and moderately resistant or susceptible types seem to show an intermediate type of resistance. With respect to the inheritance of other disease resistance factors, there is no information. As these matters are investigated, new opportunities for research will be found.

The control of diseases that has come from the use of disease resistant varieties is more effective than could be achieved by other methods of control. Leaf spot resistant varieties, for example, achieved such a degree of disease control that benefit from application of copper fungicides could not be shown, and these relatively expensive control methods are no longer used. Similarly, no advantage has been shown in applying

insecticides to kill beet leafhoppers on young curly top resistant plants, although such might be helpful with only mildly resistant types.

Farmers are now supplied with resistant varieties and they have a larger measure of security from crop failure because of diseases than ever before. But with the success of control of sugar beet diseases, the impression should not be gained that the improved varieties have removed disease loss from the crop. Even with the best resistant varieties, there is still a measurable toll from curly top, leaf spot, black root, blight, and rust. New biotypes of old pathogens may suddenly appear and new and dangerous diseases may be introduced. Even now, virus yellows offers a new threat to our western industry. If the gains already made are to be maintained, and if the future of the industry is to be safeguarded, plant pathological and plant breeding research must continue.

SUMMARY

The sugar beet has been subjected to such severe losses from epidemic diseases—notably curly top (virus), leaf spot (*Cercospora beticola*), and black root (*Aphanomyces cochlioides*)—that the very existence of the beet sugar industry in affected districts was threatened. Breeding programs have developed varieties resistant to the major diseases so that losses in great measure are prevented. A seed enterprise produces enough seed of disease resistant sorts to meet the national requirements, eliminating the need for importations from Europe. The factors for resistance to downy mildew (*Peronospora schachtii*) and rust (*Uromyces betae*) have been combined with factors for resistance to curly top; resistance to leaf spot (*Cercospora beticola*) and to black root (*Aphanomyces cochlioides*) have been combined. Breeding research is now seeking to combine these factors for disease resistance with improved physiological and morphological characters.

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STUDIES ON A MOSAIC OF NASTURTIUM OCCURRING IN BRAZIL [1]

Karl Silberschmidt

In recent years several virus diseases of nasturtium (*Tropaeolum majus* L.) have been described. Smith 1949 (6) has pointed out that this species is susceptible to infection with tomato spotted wilt virus, which induces circular necrotic spots on leaves and stunting of the plant. The same paper contains a brief description of a ringspot disease of *Tropaeolum*, characterized by distortion and mottling of leaves, some necrosis and stunting of the plant. The virus responsible was shown to have a wide host range, including cucumber, broad bean, tomato and *Petunia*. The following year, the same author (7) described a mosaic-disease of *Tropaeolum*, the symptoms of which consist of a brighter mottling with less distortion of the plant than that induced by the ringspot disease. In the same year Jensen (3) described nasturtium mosaic occurring in California. The symptoms, which previously have been confused with those produced in nasturtium by tomato spotted-wilt, include veinlet clearing of the youngest leaves followed by a mosaic mottle of variable pattern and some crinkling of the leaves. The host range was shown to be limited, and the thermal inactivation point lay at 55° C. More recently Smith (8) has studied a virus disease of *Chrysanthemum* related to cucumber mosaic which induces symptoms also in *Tropaeolum*, *Zinnia*, *Soja*, *Phlox* and *Lactuca*.

In our studies, started in 1949, we tried to find out whether or not a relationship exists between the *Tropaeolum* mosaic virus, occurring in São Paulo, Brazil, and the causal agents of diseases of that plant described elsewhere.

SYMPTOMS ON NATURALLY INFECTED NASTURTIUM PLANTS.—The symptom pattern displayed by naturally infected plants of *Tropaeolum majus* includes many different characteristics, several of which may be found on the same plant.

In recently infected plants there often is a veinclearing, ruffling, and cupping of the young leaves. In later stages of the disease, the veins often are milky colored (Fig. 1 A) and a dark-green veinbanding appears. This banding may occur in small areas scattered over the leaf or may form large arrowshaped blotches (Fig. 1 B) at the distal ends of the large veins. In such cases, the tissue between the veins and near the margin of the leaf may become chlorotic or necrotic. In other leaves, the areas between the veins show circular chlorotic spots with white necrotic rings or half rings (Fig. 1 C). There is some mottling of young leaves and older leaves may have ruffled margins.

Flowers of affected plants may be small and show a crinkling of the petals. There also is a color "break" in the petals consisting of small, irregular, yellow spots about 1 mm. in diameter. The lobed borders of these spots are a darker orange color and the remainder of the petal is darker than the center of the spots. Sometimes, the veins are abnormally dark in color and stand out against the lighter interveinal tissue.

Many infected plants do not show breaking in the color of the flowers. This suggests that breaking depends on some additional factor such as the stage of development at which the plant is infected.

SYMPTOM EXPRESSION ON EXPERIMENTALLY INFECTED NASTURTIUM PLANTS.—Sap of diseased plants (1 gm. of tissue in 1 cc. of dist. water) was rubbed over the carborundum-powdered surface of very young nasturtium plants grown in our greenhouses. As early as 5–7 days after the inoculation we observed a generalized clearing of all the veins of the 2nd and 3rd order (Fig. 1 E). A few days later, the clearing became more pronounced, but was limited to veins of areas near the margin (Fig. 1 F). The new leaves formed in the meantime, often malformed and cupped, were almost transparent and of a slightly olive color (Fig. 1 G). In those leaves the clearing of veins was no longer conspicuous. About 2 weeks after inoculation the leaves which first showed clearing of veins displayed a pattern consisting of malformation, veinbanding and necrotic veinclearing.

On the inoculated leaves we often observed yellow circular blotches or irregular lesions scattered over the leaf surface (Fig. 1 H). Frequently they grew until large areas of the leafblade, principally along the veins, turned yellow (Fig. 1 I). Sometimes the borderline of the lesions was necrotic and showed a dark color (Fig. 1 J). Since the lesions on the inoculated leaves generally appeared after the first systemic symptoms on young leaves and since similar yellow blotches could occasionally be observed on old systemically infected leaves, we believe that these local symptoms are not the result of localized penetration of virus particles into the leaf.

DISTRIBUTION OF THE VIRUS WITHIN THE INOCULATED NASTURTIUM PLANT. — Since systemic symptoms of *Tropaeolum* mosaic always appear only in the young leaves, we sought to determine whether or not virus from the inoculated leaf moves to the roots. From 4 nasturtium plants which had been inoculated 27 days before with *Tropaeolum* mosaic, we collected samples of roots (washed and superficially dried), shoots and leaves. Each of the samples consisted of 2.4 gm. of fresh tissue. The saps prepared in the usual way from these samples were used to inoculate respectively 3 batches of 4 plants. The sap from the root-sample did not induce the disease in the inoculated plants, whereas 3 of the plants inoculated with the shoot-sap after due time displayed slight, and 1 plant showed severe, symptoms of *Tropaeolum* mosaic. The 4 plants inoculated with the leaf sample exhibited severe symptoms.

In a second experiment performed March 28, 1952 with plants inoculated 11 days before, saps were prepared from 1) roots, 2) basal 10 cm. of shoot, 3) me-

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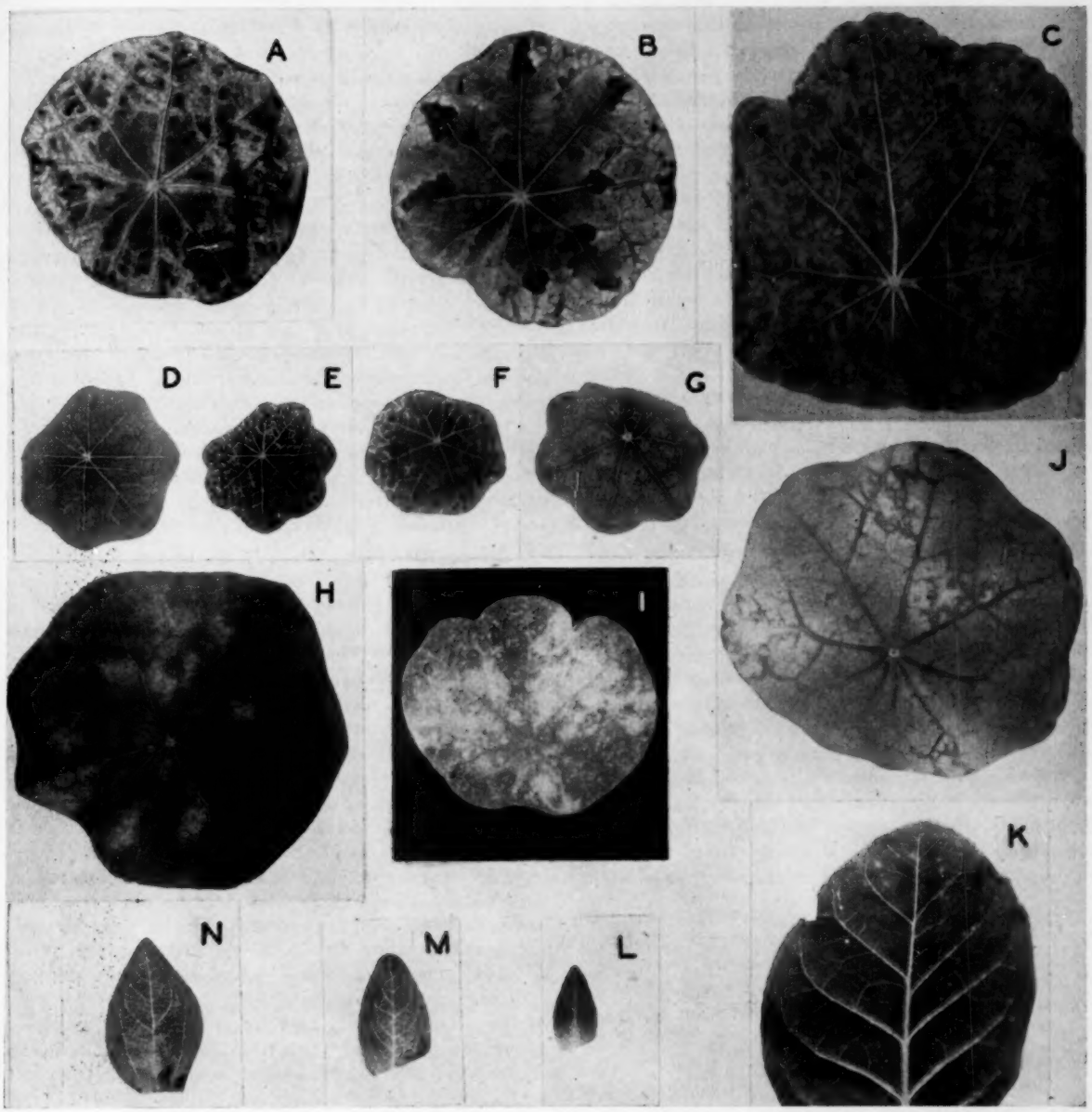

Fig. 1. A-C) Symptoms exhibited by leaves of spontaneously infected plants of *Tropaeolum*; A) milky veinclearing; B) veinbanding in form of dark-green arrows; C) chlorotic spots and rings between the secondary veins; D) young leaf of healthy nasturtium plant. E-G) systemic symptoms on leaves of nasturtium plants experimentally infected with Tropaeolum mosaic; E) generalized clearing of the veins; F) clearing of the marginal veins; G) chlorosis and transparency of young leaf. H-J) local blotches on leaves of experimentally infected plants; H) distinct chlorotic spots within the green leaf area; I) coalescent yellow blotches on old leaf; J) irregular chlorotic spots limited by brown, necrotic borderline; K) local symptoms on leaves of *N. Tabacum* "White Burley," inoculated with Tropaeolum mosaic; L) healthy young leaf of *Zinnia elegans;* M) veinclearing in *Zinnia*, recently infected with Tropaeolum mosaic; N) chlorotic blotches on the leaf base of *Zinnia*, experimentally infected by Tropaeolum mosaic.

dium 10 cm. of shoot including a small part of tissue situated above the inoculated leaf, 4) petioles of the leaves arising from the shoots below the inoculated leaf, 5) leaves corresponding to the petioles mentioned under 4, 6) apical part of the shoot, 7) petioles of the apical leaves, 8) apical leaves, 9) sepals of a flower, 10) petals of a flower. The first 5 samples consisted of 1 gm. of fresh tissue each, the others of less. The quantity of water added to prepare the sap was always proportional to the weight of the sample. Each sample was used to inoculate 4 plants.

The saps prepared from 1), 2), 4) and 5) did not cause infection. The saps from 3), 6), 7), 8), 9), and 10) induced symptoms in the inoculated plants. It is concluded that the virus had moved upward, not downward, from the inoculated leaf.

INOCULATION TESTS ON OTHER PLANT SPECIES.—In repeated inoculations with leaf extracts we were unable to induce disease symptoms in plants of *Oxalis* sp., *Lactuca sativa*, *Pelargonium hortorum*, *Capsicum annuum*, *Petunia hybrida*, and *Nicotiana rustica*. Neither did we succeed in infecting *Lycopersicum esculentum* by mechanical methods or by grafting healthy tomato plants with mosaic-diseased scions of *Tropaeolum majus*.

Among the many young plants of *N. tabacum* var. "White Burley" and *N. glutinosa*, which we inoculated with *Tropaeolum* mosaic virus, a few showed indeterminate round, yellowish spots on the inoculated leaves. In the tobacco plants these circular spots were situated in general near the leaf tip (Fig. 1, K). None of these plants developed systemic disease symptoms. Neither did tobacco plants grafted with mosaic-diseased nasturtium scions display symptoms. With juice extracted from the indefinite, local lesions produced on leaves of *N. glutinosa* inoculated with *Tropaeolum* mosaic, we induced symptoms in only one of 4 *Tropaeolum* plants, but in none of 4 tobacco plants.

Zinnia elegans Jacq. is the only species so far besides *Tropaeolum majus* L., in which we could experimentally induce a systemic disease with *Tropaeolum* mosaic virus. About 10 days after inoculation of *Zinnia* plants (var. "Lilliput Golden Gem," "Lilliput White," or "Polar Bear") with *Tropaeolum* mosaic, the new leaves began to exhibit a general clearing of veins (Fig. 1, M). A few days later large irregular chlorotic areas (Fig. 1, N) appeared near the bases of these leaves (and somtimes only on ½ of the blade). Later, young and medium leaves displayed a fine mottling, consisting of small round chlorotic circles. Old leaves occasionally showed a faint veinbanding near the tip.

The symptoms induced in *Zinnia elegans* by *Tropaeolum* mosaic are different from those produced in the same species by cucumber mosaic. In plants inoculated with cucumber mosaic, almost the whole area of the young leaves becomes pallid green and only a few great blotches of dark tissue stand out, in the form of blisters. The dark areas in leaves of plants of *Zinnia elegans* infected with *Tropaeolum* mosaic virus rise less above the leaf surface. Breaking of the flowers in *Zinnia* may be caused by *Tropaeolum* mosaic virus as well as by cucumber mosaic virus. In plants infected with *Tropaeolum* mosaic virus the petals show many faint narrow longitudinal stripes of darker color. In plants of the same species infected with cucumber mosaic virus the breaking is more pronounced. In this case the longitudinal bands of dark color, larger than in flowers of nasturtium plants infected with *Tropaeolum* mosaic, are close to the margin of the petals, the center of which displays a paler color.

Relation between Tropaeolum mosaic virus and Lycopersicum virus 3.—Smith (6) and Jensen (3) state that nasturtium plants are susceptible to *Tropaeolum* mosaic virus as well as to *Lycopersicum* virus 3. Jensen points out expressly that the symptoms of a virus disease of *Tropaeolum majus* L. have previously been confused with those produced in nasturtium by tomato spotted wilt.

Since spotted wilt is very common in Brazil, on tomato, tobacco, and other plants, we wanted to learn the exact type of symptoms induced by that virus in *Tropaeolum* plants and to compare it with the symptom pattern characteristic for *Tropaeolum* mosaic.

The descriptions of various authors regarding symptoms induced by tomato spotted wilt in nasturtium plants do not entirely agree and are only of relative value in establishing the differences between the diseases caused in nasturtium by *Lycopersicum* virus 3 and by *Tropaeolum* mosaic virus. This is especially true regarding the description, which Smith (5) gives of the symptoms caused in nasturtium by *Lycopersicum* virus 3. The symptoms, he described coincide largely with those we observed on nasturtium plants infected with *Tropaeolum* mosaic virus. Also Fig. 48 in Smith's textbook showing a leaf of Tropaeolum with numerous necrotic spots could easily represent a leaf of a plant of *Tropaeolum* infected with *Tropaeolum* mosaic.

Among the other authors, who mention *Tropaeolum majus* as host plant of the virus of spotted wilt, Costa and Forster (1) were unable to induce symptoms in tobacco by inoculation with extract of a plant of nasturtium supposed to be spontaneously infected with *Lycopersicum* virus 3. Also Norris (4) in his very detailed description of symptoms induced in *Tropaeolum majus* by various strains of tomato spotted wilt virus, found that mild and very mild strains of this virus either failed to produce observable symptoms in nasturtium or induced only a faint mottle of young leaves. Nasturtium plants infected by Norris with severe strains of spotted wilt virus exhibited necrotic local lesions and a strong veinal yellowing of the young leaves. Delle Coste and Zabala (2) quoted vein necrosis of the tender leaves and a general mottling characterized by sparse necrotic lesions as symptoms of the disease caused by spotted wilt virus in *Tropaeolum*.

Since inaugurating those studies in 1949, we have made about 35 successive transfer tests, always inoculating by extracted juices (prepared with 0.1 per cent sulphanilamide and 0.2 per cent sodium sulfite) batches of new plants with virus obtained from infected leaves of earlier series. In the course of these studies we made use of several sources of the virus of spotted wilt. Two of the sources came originally from tomato plants naturally infected in the field, 2 others from tobacco plants displaying symptoms of natural infection. In the course of these transfer tests 127 of 173 tobacco plants and 93 of 151 plants of *Nicotiana glutinosa* inoculated by sap with *Lycopersicum* virus 3 came down with typical symptoms of spotted wilt. None of 59 *Tropaeolum* plants inoculated with the same virus showed any distinct symptoms. We have failed in several trials to recover the virus of spotted wilt from *Tropaeolum* plants, previously inoculated with this virus. From our results we conclude that *Lycopersicum* virus 3, or at least the strains of that virus occurring in Brazil, when inoculated by mechani-

cal methods do not normally induce a systemic disease in nasturtium.

BEHAVIOR OF THE VIRUS OF TRAPAEOLUM MOSAIC IN VITRO.—*Experiments on the thermal inactivation point of Tropaeolum mosaic.*—To determine the thermal inactivation point of *Tropaeolum* mosaic virus, we performed 12 series of experiments, using various sources of the virus and repeating the tests in successive months. Infectious saps were placed in thin-walled test tubes closed with rubber stoppers and immersed in a waterbath for 10½ minutes. From the results it was concluded that the thermal inactivation point is 58° C.

Some strains were inactivated at 56°C. Saps from old leaves were more easily inactivated than those from young ones.

Experiments on dilution end-point and longevity in vitro of Tropaeolum mosaic.—We observed in some of our experiments that the infective activity of *Tropaeolum* mosaic virus drops considerably even after moderate dilution. In the few tests specially performed to determine the dilution end-point, infection was caused by juices of diseased plants diluted with 100 parts of water, but not at dilutions of 1–1000 and 1–10,000.

With regard to longevity *in vitro* we found, that juice extracts of mosaic-diseased *Tropaeolum* plants, when kept in a refrigerator (3° C), were still infective after 72 hours, but when held at room temperature (25° C) lost infectivity within 24 hours.

STRAINS OF TROPAEOLUM VIRUS.—Since great differences in symptom-expression exist among naturally mosaic-diseased *Tropaeolum* plants, we wanted to find out if these differences in symptom patterns could be attributed to the existence of virus strains. Therefore we selected 9 individual plants exhibiting different symptom-types and performed successive inoculations with the extracted juices of each of these plants. In general, the symptoms predominant in the source plant (f. i. crinkling) was not consistently reproduced in the representatives of the transfer-tests.

On the other hand, the degree of severity of symptoms caused by juices extracted from individual source plants remained rather constant in the course of successive transfers. This observation made us believe in the existence of severe and of mild strains of the *Tropaeolum*-mosaic virus.

The juice extracted from our source plant M.M., f.i., in successive transfer tests, induced the yellow blotches on the inoculated leaves described in this paper, a consistent stunting of the whole plant and the development of very small crinkled and cupped young leaves. We consider these symptoms as characteristic for severe strains of *Tropaeolum*-mosaic-virus.

Extracts of most of the other source plants induced only a few scattered chlorotic spots on the young leaves, which, however, later on expanded normally, exhibited a smooth surface, and reached considerable size. Those source plants were considered to harbor mild strains of *Tropaeolum* mosaic virus.

Cross-protection tests between various strains of Tropaeolum mosaic virus.—We performed a few experiments to determine whether *Tropaeolum* plants infected with a mild strain of the mosaic virus are protected from the effect of super inoculation with a more severe strain of the same virus. For this purpose young leaves, displaying very slight systemic symptoms of infection, were inoculated with juice containing our most severe strain (M.M.). At the same time young leaves of healthy check plants were rubbed with the same virus-containing juice. After a week the check plants showed yellow blotches on the inoculated leaves and severe systemic symptoms on the young leaves. The leaves of the "premunized" plants continued displaying only a very slight mottling.

DISCUSSION.—Results of our experiments show clearly that the virus producing the mosaic disease of *Tropaeolum* in São Paulo and the causal agent of spotted wilt of tomato are not identical.

Comparing the mosaic disease of *Tropaeolum* occurring in São Paulo with the diseases of ornamental species described by other authors, we may immediately disclaim the identity between the Brazilian *Tropaeolum* disease and *Tropaeolum* ringspot, as described by Smith (7). According to the observation of Smith, the virus causing *Tropaeolum* ringspot "has a wide host range and will infect a variety of ornamental plants including the Petunia." In our experiments *Petunia* was not infected by Brazilian *Tropaeolum* mosaic. Neither did our virus induce ringspot symptoms in the host plants. In the beginning of our studies we considered the possible existence of a certain relation between *Tropaeolum* mosaic virus and cucumber mosaic virus. Our *Tropaeolum* virus, however, did not infect *Lactuca sativa* and other species quoted by Smith as host plants of cucumber mosaic virus. Therefore we do not believe that our *Tropaeolum* mosaic has any relation to that described recently by Smith (8) as being caused by cucumber mosaic virus. Incidentally we learned from the results of some of our experiments that at least our strain of cucumber mosaic virus did not infect *Tropaeolum* plants.

There remain 2 more diseases to be compared with our *Tropaeolum* mosaic. One of them is *Tropaeolum* mosaic as described by Smith (7), the other is nasturtium mosaic, studied by Jensen (3). The symptoms of Smith's *Tropaeolum* mosaic, as far as can be judged from the photograph illustrating his paper, are similar to those of the Brazilian *Tropaeolum* mosaic. But we could never, except in the case of a single plant possibly contaminated by another virus, induce systemic symptoms in *Nicotiana glutinosa* with our virus, whereas in Smith's tests the inoculated plants of *N. glutinosa* displayed symptoms resembling those caused by the cabbage black ringspot virus.

There still remains to be considered the nasturtium mosaic described by Jensen. It is true that between the virus used in our experiments and Jensen's nasturtium mosaic virus, some differences exist with regard to their behavior *in vitro*. We found the thermal

inactivation point at 58° rather than at 55°, as Jensen did. On the other hand, the virus described by Jensen stood higher dilutions (1: 20,000), without losing its infectivity, than did our virus (1: 100). But since results of tests on the qualities of viruses *in vitro* depend, to a certain extent, on the virus concentration of the inoculum, the number of inoculated plants and the methods which have been used, we do not think we should attribute too great an importance to these differences. The symptom pattern described by Jensen coincides in many points with the characteristics which we found in nasturtium plants infected with our *Tropaeolum* mosaic. We can not go any further in this comparison, since Jensen in his short note on nasturtium mosaic does not quote the name of the species experimentally inoculated with his virus. We therefore do not know if he has performed experiments with *Zinnia*. Even so, we believe that Jensen's nasturtium mosaic and our *Tropaeolum* mosaic have many characteristics in common.

SUMMARY

Nasturtium plants grown commonly in São Paulo, Brazil, for ornamental purposes often display symptoms of a mosaic disease characterized by mottling, veinbanding, crinkling of the leaves, and in extreme cases by dwarfing of the whole plant (Fig. 1, A, B, C.). The disease is transmissible by plant extracts.

In nasturtium plants the *Tropaeolum* mosaic virus moves readily upward, but not downward. In systemically infected plants no virus could be detected in roots. The natural spread of infection in flower beds does not seem to occur through the roots.

Among the various plant species experimentally inoculated with *Tropaeolum* mosaic virus, in addition to nasturtium, only *Zinnia elegans* displayed symptoms of a systemic disease (Fig. 1, M). The inoculated leaves of *Nicotiana tabacum*, *Nicotiana glutinosa* and *Lycopersicum esculentum* sometimes exhibited a few gray sunken spots, but the disease never became systemic in those plants (Fig. 1, K).

With *Lycopersicum* virus 3 (the virus of tomato spotted wilt)—in spite of extensive attempts—we did not succeed in inducing a systemic disease in *Tropaeolum* plants. *Tropaeolum* mosaic virus is not identical with *Lycopersicum* virus 3. The strains of *Lycopersicum* virus 3 occurring in São Paulo do not normally induce the severe symptoms in *Tropaeolum majus* described by Norris, at least in tests performed with mechanical methods.

The thermal inactivation point of *Tropaeolum* mosaic virus was found to be 58° C. In a limited number of tests, juice extracted from diseased plants lost its infective activity when diluted with more than 100 parts (in volume) of water. Also undiluted juice kept 24 hours or more at room temperature was found noninfective.

Juices extracted from different source plants showed consistent differences in relation to the severity of symptoms they induced in inoculated plants, indicating the existence of severe and mild strains of *Tropaeolum* mosaic-virus.

Plants systemically infected by a mild strain of *Tropaeolum* mosaic were entirely or partly resistant to superinoculation by a severe strain of the same virus.

The *Tropaeolum* mosaic described has many characteristics in common with Jensen's nasturtium mosaic.

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HOST RANGE AND EPIPHYTOLOGY OF THE CEREAL YELLOW DWARF DISEASE[1]

John W. Oswald[2] and Byron R. Houston[2]

The yellow-dwarf disease of cereal crops which was epiphytotic in California in 1951 (5) is characterized by a brilliant leaf yellowing of barley, reddening of oats, and chlorosis of wheat. Moderate to severe stunting occurs on all 3 cereals. Investigations (2, 3, 5) have shown that the disease is caused by a virus that is readily transmitted by aphids; all attempts to transfer the yellow-dwarf virus by mechanical means, by seed or in the soil have been negative. All 5 grain-infesting aphid species tested—English grain aphid, *Macrosiphum granarium* (Kirby), grass aphid, *M. dirhodum* (Walker), corn aphid, *Rhopalosiphum maidis* (Fitch), apple-grain aphid, *Rhopalosiphum prunifoliae* (Fitch), and the greenbug, *Toxoptera graminum* (Rondani)—have proven to be efficient vectors of the yellow-dwarf virus.

After proof of the virus nature of cereal yellow-dwarf and the indictment of aphids as vectors, the problem of explaining the sudden occurrence of this destructive disease remained. Unquestionably the disease has occurred at a low ebb in California cereals the past few years, probably for a much longer period. Scattered diseased oat and barley plants resembling yellow-dwarf have been noted since 1947. Suddenly in mid-April of 1951 yellow-dwarf became prevalent on all 3 major cereals in most grain producing areas of the state and caused severe damage, particularly to barley.

This paper deals primarily with an investigation of the host range of the cereal yellow-dwarf virus in the grass family (4). This information is used together with a knowledge of the climatic conditions that prevailed in 1951 and 1952, in an effort to gain an understanding of the epiphytology of yellow-dwarf.

MATERIALS AND METHODS.—Plants of each grass species to be tested for reaction to yellow-dwarf were grown in 2 6-in. pots in the greenhouse, and at the 2–3 leaf stage thinned to 5 plants per pot. A few of the perennial species, such as Bermuda grass, *Cyonodon dactylon* (L.) Pers., were started with rhizomes rather than true seed. Apple-grain aphids (*R. prunifoliae*) which had been reared on barley plants infected with yellow-dwarf were transferred to the grasses with a camel's hair brush. Ten aphids were placed on each of 10 seedlings of each grass and the pots covered with muslin cages for a 3-day aphid feeding period. Before removing the aphids with a 1 per cent parathion spray, the survival of aphids on each grass was checked. A few grasses were encountered for which a satisfactory test could not be completed because none of the vectors could be induced to feed satisfactorily. For use as noninoculated checks, 2 pots of each grass were similarly caged without aphids for the 3-day period. With each series of grasses tested, viruliferous aphids were placed on 10 seedlings of the susceptible barley variety, Blackhulless (C.I. 666) in order to check the infectivity of the aphid colonies employed. In every series all such barley plants subsequently developed typical yellow-dwarf.

After the cages were removed, the pots were held in the greenhouse at 24°–28°C. and watered only with distilled water. Yellow-dwarf appeared on all grasses that expressed symptoms, at approximately the same time as on the susceptible barley—14–21 days following aphid inoculation.

An attempt to recover the yellow-dwarf virus from each grass species was made approximately 1 month after inoculation. Non-viruliferous apple-grain aphids were caged on the grasses, allowed to feed 3 days, and then transferred to 5 Blackhulless barley seedlings (10 aphids per plant) for a 3-day feeding period. Recovery was considered positive if yellow-dwarf subsequently developed in the barley. Aphids used in every recovery series were proven to be nonviruliferous by placing others from the same colonies on Blackhulless barley seedlings without a feeding interval on one of the grasses.

HOST RANGE OF THE YELLOW-DWARF VIRUS IN THE GRAMINEAE.—Fifty-five species of grasses[3] were tested as described above for their reaction to the cereal yellow-dwarf virus. The grasses were selected so as to include many of the most common annual and perennial species of California and at the same time to have representatives from a majority of the tribes of the Gramineae. On the basis of these inoculation studies the grasses could be divided into 3 groups: 1) 20 exhibiting symptoms of yellow-dwarf and from which the virus could be recovered (Table 1); 2) 16 that exhibited no symptoms but from which the yellow-dwarf virus could be recovered; and 3) 19 grasses that proved to be immune, i.e., showed no symptoms and from which the virus could not be recovered.

A large majority of the grasses in the group showing symptoms were widespread winter-growing annuals of California such as annual bromes (Fig. 1), annual fescues, wild barleys and wild oats (Fig. 2).

Sixteen grass species developed no symptoms following inoculation with the yellow-dwarf virus, but recovery trials showed them to be symptomless carriers. These grasses are as follows: Tribe FESTUCEAE—*Dactylis glomerata* L. (Orchard grass), *Festuca arundinacea* Schreb. (Kentucky Fescue 31), *Poa annua* L. (Annual bluegrass), *Poa pratensis* L. (Kentucky bluegrass); Tribe HORDEAE—*Agropyron trachycaulum*

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TABLE 1.—*Grasses that exhibited symptoms following aphid inoculation in the seedling stage with the cereal yellow-dwarf virus and from which the yellow-dwarf virus could be recovered* [a,b]

Tribe	Species	Symptoms
FESTUCEAE		
	Bromus catharticus Vahl. (Rescue grass) P[c]	leaf reddening, moderate stunting
	" *inermis* Leyss. (Smooth Brome grass) P	leaf serration, severe stunting
	" *mollis* L. (Soft chess) A	extreme stunting—no pigment change
	" *rigidus* Roth. (Ripgut) A	leaf purpling—moderate stunting
	" *rubens* L. (Red bromegrass) A	" " " "
	" *tectorum* L. (Downy chess) A	" " " "
	Cynosurus echinatus L. (Dogtail) A	slight stunting
	Festuca myuros L. (False foxtail fescue) A	moderate stunting
	" *reflexa* Buckl. (Annual fescue) A	extreme stunting-leaf and crown reddening
HORDEAE		
	Aegilops triuncialis L. (Barb goatgrass) A	brilliant leaf purpling-mod. stunting
	Hordeum hystrix Roth. (Mediterranean barley) A	leaf purpling—severe stunting
	" *leporinum* Link. (Annual wild barley) A	leaf yellowing—no stunting
	" *brachyantherum* Nevski. (Meadow barley) P	moderate stunting
	Sitanion hystrix (Nutt.) J. G. Smith (Squirreltail) P	slight leaf yellowing only
AVENEAE		
	Avena barbata Brot. (Slender wild oat) A	leaf reddening—moderate stunting
	" *fatua* L. (Wild oat) A	" " severe "
AGROSTIDEAE		
	Aristida oligantha Michx. (Prairie three-awn) A	leaf purpling—slight stunting
	Gastridium ventricosum (Gouan) Schinz and Thell. (Nitgrass) A	leaf reddening—severe stunting
PHALARIDEAE		
	Phalaris parodoxa L. (Canary grass) A	moderate to severe stunting
ANDROPOGONEAE		
	Andropogon barbinodis Lag. (Cane beard grass) P	leaf reddening—slight stunting

[a] Ten viruliferous apple-grain aphids (*Rhopalosiphum prunifoliae*) were placed on each of 10 seedlings of each grass and allowed to feed for 3 days.

[b] Non-viruliferous apple-grain aphids were placed on each grass approximately 1 month after inoculation with yellow-dwarf and allowed to feed for 3 days. Then 10 aphids were transferred to each of 5 Blackhulless barley seedlings to determine if the virus could be recovered.

[c] P indicates perennial; A, annual.

(Link) Malte. (Slender wheat-grass), *Elymus caput-medusae* L. (Medusahead), *Elymus triticoides* Buckl. (Beardless wild-rye), *Lolium multiflorum* Lam. (Annual rye-grass); Tribe PHALARIDEAE—*Phalaris tuberosa* L. (Harding grass), *Anthoxanthum odoratum* L. (Sweet vernalgrass); Tribe CHLORIDEAE—*Bouteloua curtipendula* (Michx.) Torr. (Side-oats gramagrass), *Chloris gayana* Kunth. (Rhodesgrass), *Cynodon dactylon* (L.) Pers. (Bermuda grass); Tribe PANICEAE—*Panicum capillare* L. (Witchgrass); Tribe ANDROPOGONEAE—*Sorghum sudanense* (Piper) Stapf. (Sudan grass), *Sorghum vulgare* Pers. (Double Dwarf 38 milo). Among these were orchard grass and Kentucky fescue 31 both of which grow throughout the summer in California as important components in irrigated pastures. Others such as Bermuda grass and Kentucky blue grass are omnipresent in lawns. Of the summer cultivated crops, Double Dwarf 38 milo and Sudan grass are symptomless suscepts.

Only 19 of the 55 grasses appeared to be immune from yellow-dwarf: Tribe HORDEAE—*Agropyron cristatum* (L.) Gaertn. (Crested wheat grass), *Elymus glaucus* Buckl. (Blue wild-rye), *Lolium perenne* L. (Perennial rye grass 12); Tribe AVENEAE—*Arrhenatherum elatius* (L.) Presl. (Tall oatgrass), *Deschampsia danthonioides* (Trin.) Munro ex Benth. (Annual hair grass), *Holcus lanatus* L. (Velvet grass); Tribe AGROSTIDEAE—*Agrostis alba* L. (Redtop), *Agrostis exarata* Trin. (Spike bent), *Alopecurus pratensis* L. (Meadow foxtail), *Oryzopsis hymenoides* (Roem. and Schult.) Ricker. (Indian ricegrass), *Phleum pratense* L. (Timothy), *Stipa cernua* Stebbins and Love. (Nodding needlegrass; Tribe CHLORIDEAE—*Bouteloua gracilis* (H.B.K.) Lag. ex Steud. (Blue gramagrass); Tribe PANICEAE—*Digitaria sanguinalis* (L.) Scop. (Crabgrass), *Echinochloa crusgalli* (L.) Beauv. (Water or barnyard grass), *Paspalum dilatatum* Poir. (Dallis grass), *Setaria viridis* (L.) Beauv. (Green bristlegrass); Tribe ANDROPOGONEAE—*Sorghum vulgare* Pers. (Double Dwarf white sooner milo); Tribe TRIPSACEAE—*Zea mays* L. (Golden cross Bantam corn).

EPIPHYTOLOGY OF CEREAL YELLOW-DWARF.—In California spring varieties of barley, wheat, and oats are grown as winter crops. Cereals are planted after the weeds and volunteer plants (if following grain) which are brought up by the first fall rains can be worked under and the land made ready. Ordinarily in the central valley most grain is seeded in December. Jan-

FIG. 1. Effect of the cereal yellow-dwarf virus on soft chess (*Bromus mollis*). Left, 5 non-inoculated plants, 3 months old; right, 5 plants 3 months after inoculation at the seedling stage with viruliferous aphids.

uary, and into February. The date of planting varies with the season depending to a great extent upon rainfall. Wheat and oats are rarely planted much later than February 15, but barley often is seeded in March and into April. The discussion of the development of cereal yellow-dwarf in the 1950-51 and 1951-52 seasons in California is confined primarily to the Sacramento Valley and northern San Joaquin Valley in which cereals, particularly barley, are widely grown. The climate at Davis[4] where the weather data were taken, is roughly representative of this grain producing area.

1950-51 season.—During the winter and spring of 1950-51, rainy, cloudy weather was practically continuous from the end of October 1950 through the first week in March, 1951. As a result soils remained too wet for plowing or weed cutting and thus only a small acreage of grain was planted by March 1. Insofar as yellow-dwarf was concerned, the continuous rainy but abnormally warm weather had 3 effects: 1) it delayed planting of cereals; 2) it encouraged a rank growth of winter annual grasses; and 3) it was favorable for a heavy build-up of aphids on the wild grasses, which must have taken place in the light of subsequent events.

On March 7, 1951, the rainy weather halted abruptly and no rain fell until April 25, the longest early spring drought on record for the Sacramento Valley. Seeding made rapid progress and much of the grain, mostly barley, was planted by March 20. Lack of rainfall coupled with drying winds brought about a mass desiccation of the wild grasses, by the end of March, which rendered them unfavorable to the aphid populations they were supporting. Consequently, during the last week in March and the first week in April, large flights of aphids from the drying grasses into the young grain fields must have occurred. Aphid flights were observed by the authors during this period but no attempts were made to identify the species involved.

On April 11, the first infected barley plants were observed at Davis and within the next 2 weeks the disease had become epiphytotic in the Sacramento Valley and northern San Joaquin Valley. During this period aphid populations on grain were extremely heavy. As shown in previous work (5) the incubation period after inoculation with the yellow-dwarf virus is 2–3 weeks. Without doubt the sudden widespread appearance of yellow-dwarf in mid-April could be traced directly to a migration of aphids in late March from the drying grasses into the grain fields. Since a majority of the winter grasses are possible hosts of the virus, it is understandable why a large percentage of the aphids were viruliferous.

It has been emphasized (5) that the severity of symptoms caused by the yellow-dwarf virus and its ultimate effect on yield are directly correlated with the growth stage of the plant when infection occurs. In the large acreage of the late-planted grain, predominately barley, the plants were in the seedling to tillering stage when infection took place. Therefore maximum damage from yellow-dwarf occurred during this season. Root

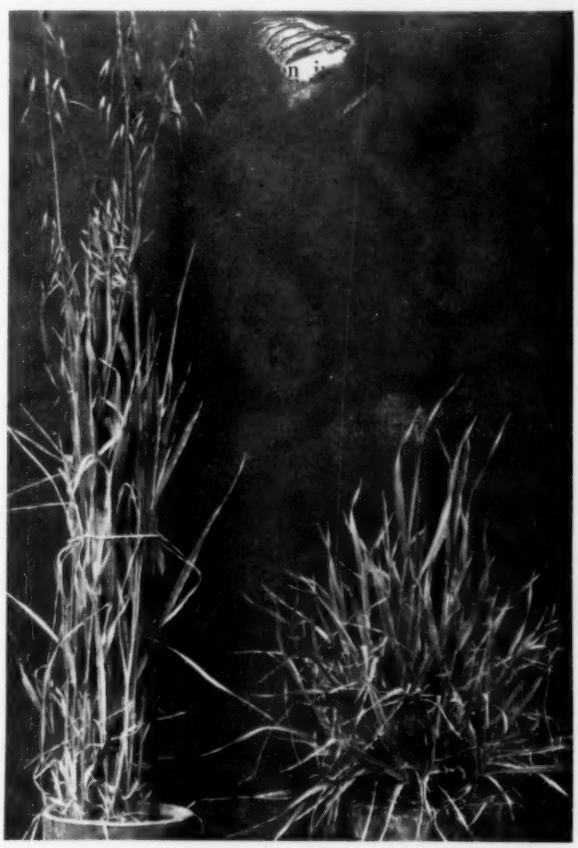

FIG. 2. Cereal yellow-dwarf virus on wild oats (*Avena fatua*). Left, 5 non-inoculated plants 4 months old; right, 5 plants 4 months after inoculation at the seedling stage with viruliferous aphids. Extreme stunting and leaf reddening are the principal symptoms.

[4] Climatological data obtained from the Department of Irrigation, University of California, Davis, California and from the U. S. Department of Commerce, Climatological Data—California.

development of infected plants is equally as retarded as the top growth, and the drought that occurred further accentuated the situation. In the volunteer fields and the few fields of grain planted in November or December, infection with yellow-dwarf was nearly 100 per cent, but damage was slight. Such grain was in the heading rather than seedling stage when infection occurred and the disease was apparent only as a yellowing (barley and wheat) or reddening (oats) of the uppermost leaves.

1951-52 season.—In this growing season, although total rainfall far surpassed that of the previous one, yellow-dwarf was not nearly so important. Fall rains were heavier than normal but in December there occurred a 20 day period of dry clear weather. After the experience of the preceding season, most of the grain growers took advantage of this opportunity to plant and as a result a large acreage of cereals was seeded by January 1, 1952. Climatic conditions through March prevented the sudden drying of grasses as occurred in 1951 and were unfavorable for rapid aphid reproduction. An aphid build up did occur on wild grasses in early April and naturally infected plants were common. Flights of consequential size from wild grasses to grain fields must have taken place about April 15-20 as indicated by the widespread outbreak of yellow-dwarf during the first week of May, 1952. Damage was slight, however because most grain had been planted in December and was at the heading stage when infection occurred in mid-April. The few barley fields that had been planted in late March were in the seedling stage when infected, and in some of these, damage was so severe they were abandoned.

Discussion.—It appears after 2 years of observation that the cereal yellow-dwarf disease will be of economic importance only in those years that climatic conditions during the winter and spring would 1) delay planting of cereals until March; 2) induce a rank cover of wild grasses on the ranges and in the cultivated areas; 3) encourage multiplication of the aphid vectors of the virus on the susceptible wild grasses; 4) bring about a rapid drying of the grasses soon after the grain crops emerge. The abnormally warm but continuously wet winter of 1950-51 followed by a record spring drought from March 7-April 25 brought about all 4 of the above situations and the result was a damaging epiphytotic of yellow-dwarf. Yellow-dwarf was nearly as widespread in 1951-52, but infection of grain occurred at such a late stage of growth that damage was slight.

In the central valleys of California, yellow-dwarf will probably be of economic importance only on barley. Wheat and oats normally are seeded by February 15 and are at an advanced stage of growth when aphid flights from the grasses are likely to occur. When climatic conditions delay grain planting beyond this time, growers originally intending to plant wheat or oats often seed barley instead. There were numerous exceptions, however, in the 1950-51 season; oat and wheat fields sowed in March were badly damaged by yellow-dwarf.

In the mountainous grain-producing areas of the state, such as the Tule Lake section, yellow-dwarf may become widespread, as it was in 1952, but probably will never be of economic importance. Cereals are planted in April and May and are approaching heading by the time the weather is sufficiently warm for aphid activity.

The yellow-dwarf virus must be perpetuated through the summer in either susceptible perennial grasses growing in irrigated pastures or in volunteer grain plants that grow commonly in irrigated fields or along ditch banks. There is a possibility that such summer cultivated annual crops as milo may act as reservoirs for the virus.

There appears to be a natural sequence of the occurrence of the different aphid vectors. The apple-grain aphid (*R. prunifoliae*) is predominant during the winter and early spring followed by the English grain aphid (*M. granarium*) and the grass aphid (*M. dirhodum*) that became abundant in April and May. During the warm summer months, the corn aphid (*R. maidis*) is by far the most common aphid encountered on grains and grasses. This species is probably responsible for the summer spread of the virus on the susceptible grasses and for the infection of the young annual grasses that emerge after the first fall rains.

The eventual control of yellow-dwarf lies in the development of resistant varieties. Resistant barleys have been found for use as parental material for such a program (5). In the interim, early planting, before February, is strongly recommended. It is doubtful if a program of aphid control in grain fields would be beneficial because of the wide host range of the virus and the aphid vectors in the grass family.

SUMMARY

Inoculation studies demonstrated that the cereal yellow-dwarf virus has a wide host range in the grass family. Of 55 grasses tested, 36 species (representing 8 tribes of the Gramineae) proved to be hosts. Twenty of these grasses exhibited typical yellow-dwarf symptoms of stunting and either yellow or red leaf discoloration. Included in this group were such common grasses as annual bromes and fescues, wild barleys, wild oats, and canary grass. Sixteen species showed no symptoms but proved to be capable of carrying the virus. Widely-grown grasses that are symptomless carriers are Bermuda grass, orchard grass, Kentucky fescue, and Kentucky blue grass. California's natural winter and spring range growth is made up principally of grasses susceptible to yellow-dwarf. Also a considerable proportion of the grasses grown in summer-irrigated pastures are hosts of the virus.

Conditions favoring a damaging epiphytotic of cereal yellow-dwarf are: 1) a warm but continuously wet winter which favors rank growth of wild grasses and rapid reproduction of the aphid vectors but at the same time delays planting of grain until March; 2) a sudden long dry period after the rains which brings about rapid drying of the grasses soon after grain is

seeded; 3) large flights of aphids from the drying grasses into very young grain fields.

In the 1950-51 season when damage was severe, aphid flights from the grasses apparently occurred in late March and early April into young grain that had been planted after March. 1. Yellow-dwarf was as widespread in 1951–52, but aphid flights from the grasses came in late April and the grain which, for the most part, had been planted in December was approaching heading. Losses from infection at this stage were slight.

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THE CONTROL OF LOOSE SMUT OF BARLEY AND WHEAT BY SPERGON AND BY SOAKING IN WATER AT ROOM TEMPERATURE [1]

L. E. Tyner [2]

Tyner[3] reported that the loose smut fungus, *Ustilago nuda* (Jens.) Rostr., was controlled in varying degrees by soaking infected seed of barley in solutions of oxyquinoline sulphate, boric acid, potassium iodide, or Spergon (tetrachloropara-benzoquinone). Spergon was thought to be the most satisfactory as it produced less mortality of healthy seed than the others. Results of further experiments with Spergon, undertaken in 1951, were reported in part by Tyner and Russell.[4] Essentially complete control of loose smut of barley was obtained by extending the period of immersion in 0.2 per cent Spergon suspension to 40, 44, or 48 hours. Approximately 50 per cent control resulted from immersion in water for 46 hours at room temperature.

Various factors involved in the control of loose smut by Spergon and by water soak were further investigated in 1952. The findings indicate a practical method that the farmer may use for the control of loose smut.

LOOSE SMUT OF BARLEY.—Unless otherwise stated, treatments in these and subsequent experiments were carried out at the room temperature of the laboratory, which varied over the approximate range 72°–77° F.

Control by Spergon.—Seven lots of barley seed infected with loose smut were soaked in water for 6, 8 and 10 hours in the laboratory. After the presoak in water the seed was drained and 0.2 per cent Spergon suspension was added to cover it. The samples remained in this suspension with occasional stirring for periods of 40, 44, or 48 hours, when they were drained, dried overnight, and seeded. As a check on the action of the Spergon in controlling loose smut, other samples of the same 7 lots of seed were soaked in water for 46 hours before seeding. A third set of samples was dusted with Ceresan M before seeding. The seed was sown in the field May 17 in 12-ft. rows replicated 4 times. Two hundred seeds were sown in each row. On July 18-20 the total heads and smutted heads were counted. Data are in Table 1.

The effect of treatment on stand varied widely but only in the sample of Glacier and in one of the samples of Montcalm was the number of plants seriously reduced. Conversely, the stands of the other sample of Montcalm and of Titan were better than those of the controls. It is possible that mortality of treated seed is a function of its permeability and this factor may vary according to age of seed, conditions of growth and storage, maturity at harvest, and variety.

Loose smut was completely eliminated by 1 or more of the treatments in all samples except Titan. Smut was also reduced markedly in all samples by the 46-hour water soak. Statistical analysis of the data shows that none of the treatments controlled smut significantly better than another, and that all Spergon treatments controlled smut significantly better than did the 46-hour water soak.

Effect of temperature.—The effect of temperature of the Spergon suspension on smut control was studied. Samples of the varieties Warrior, Gem, Montcalm, Titan, and Newal infected with loose smut were treated at room temperature and at a temperature which varied between 54° and 60° F. The treatments included immersion in 0.2 per cent Spergon suspension for 48 hours, preceded by 6, 8, or 10 hours presoak in water.

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[2] Plant Pathologist.

[3] Tyner, L. E. 1951. Control of loose smut of barley by chemical and physical treatments. Sci. Agr. 31: 187-192. 1951.

[4] Tyner, L. E. and R. C. Russell. 1952. Control of loose smut in barley by tetrachloropara-benzoquinone. (Spergon-SL). U. S. Dept. Agr. Pl. Dis. Reptr. 36: 180-181.

TABLE 1.—*Control of loose smut of barley by presoaking seed in water followed by immersion in 0.2 per cent Spergon suspension at room temperature*

Treatment		Smutted Heads						
Water	Spergon	Newal[a]	Newal	Montcalm	Montcalm[b]	Titan	Plush	Glacier[b]
Hr.	Hr.	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
0	0	5.9	17.3	9.7	9.3	16.1	3.2	23.6
46	0	3.0	9.3	5.4	2.7	7.8	2.7	3.2
6	40	0.6	0.3	0	0	0.5	0.1	0
8	40	0.1	0.2	0.1	0	1.0	0.2	0
10	40	0.2	0.1	0.1	0	1.1	0.4	0.3
6	44	0.6	0.2	0	0	0.7	0	0
8	44	0.6	0.5	0	0	1.1	0	0
10	44	0.4	0.3	0	0	1.0	0	0
6	48	0	0.3	0	0	0.7	0.2	0
8	48	0	0	0	0	1.6	0.3	0
10	48	0	0	0	0	0.6	0	0

Analysis of variance (sine angle transformation)

Variation due to	*D.F.*	*M.Sq.*	*F*	d_{01}	d_{05}
Varieties	6	146.37	8.35××	±2.31	±1.78
Treatments	10	913.37	52.13××	±2.98	±2.24
T. × V.	60	17.52	2.80××		
Error	231	6.26			

[a] Newal—Bushel lot treated.

[b] Stand seriously reduced.

Controls were dry seed treated with Agrox C dust. Four randomized 12-ft. rows, 200 seeds per row, were planted in mid-May. Data are in Table 2.

Smut control was complete in all Spergon treatment schedules at room temperature in Warrior, Gem, and Montcalm, and nearly complete in Titan and Newal. Reduction in stand was important in the varieties Warrior and Gem at this temperature. On the contrary, smut control was unsatisfactory in all varieties where the temperature range was 54°–60° F.

Effect of omitting presoak period.—An experiment was designed to find whether loose smut in barley could be controlled by immersing the seed in a 0.2 per cent suspension of Spergon, omitting the preliminary presoak in water. Fort barley grown in Eastern Canada was used. Samples were soaked for periods of 48, 52, 56, or 60 hours in 0.2 per cent Spergon suspension at room temperature. Other samples were soaked in 0.2 per cent Spergon suspension for 40, 44, 48 or 52 hours after a preliminary presoak in water for 8 hours. Four randomized 12-ft. rows, 200 seeds per row, were sown. The controls were dry seed treated with Agrox C.

Excellent control of loose smut was obtained by eliminating the presoak period in water from the treatment schedule. Seed soaked in 0.2 per cent Spergon for 48, 52, 56, or 60 hours produced 0, 0, 0.1, and 0.1 per cent smutted heads, respectively. Seed soaked for 8 hours in water followed by 40, 44, 48, and 52 hours in 0.2 per cent Spergon produced 1.2, 0.3, 0.3, and 0 per cent smutted heads, respectively. Smut in control plots was 6.9 per cent. Total heads ranged from 176–231 in the rows grown from seed treated in Spergon; 232–290 in the rows grown from seed presoaked in water before immersing in Spergon suspension; and was 324 in the control rows.

Control of loose smut by water soak alone.—The effectiveness of prolonged soaking in water (Table 1), indicated further investigation of this approach. Two varieties of barley, Gem and Warrior, were soaked in water at room temperature under various conditions for periods of 48, 56, 64, 72, or 80 hours. Seed of Gem was treated in 3 ways, namely: water unchanged during the course of treatment; water changed twice daily, at 8:30 a.m. and 4:30 p.m.; and running water. There was only a slight flow of water from the tap in this latter treatment and the temperature of the water read twice daily showed little deviation from that of the laboratory. Warrior seed was treated only in standing water and in water changed twice daily. In all treatments except those where running water was used a rich bacterial flora developed and the treated seed became foul in odor as the period of immersion increased. The seed, after drying, was planted May 17 in 4 12-foot rows, 200 seeds per row. As a control, portions of the same lots of seed were treated with the mercurial dust, Agrox-C. Data are in Table 3.

The stand was satisfactory in Warrior under all treatments, but was unsatisfactory in Gem in certain cases where the duration of immersion exceeded 64 hours. Smut control in Gem was fairly satisfactory following the running water treatment, and was good in the other 2 treatments. Smut control in Warrior was complete in 5 of the treatment schedules employed. Statistical analysis of the data for Gem showed that smut control differed significantly for treatments, but

TABLE 2.—*Effect of temperature on the control of loose smut of barley by immersion in 0.2 per cent Spergon suspension*

Variety	Temperature	Treatment Schedule	Total heads	Smutted heads
	°F.	Hr.	Ave.	Per cent
Warrior	72-77	6-48	100	0
	72-77	8-48	106	0
	72-77	10-48	119	0
	54-60	6-48	287	3.2
	54-60	8-48	283	5.5
	54-60	10-48	318	4.4
	Control		330	23.8
Gem	72-77	6-48	89	0
	72-77	8-48	35	0
	72-77	10-48	40	0
	54-60	6-48	295	6.2
	54-60	8-48	320	4.1
	54-60	10-48	324	4.7
	Control		349	21.0
Montcalm	72-77	6-48	286	0
	72-77	8-48	261	0
	72-77	10-48	230	0
	54-60	6-48	354	1.8
	54-60	8-48	361	2.1
	54-60	10-48	338	1.0
	Control		361	9.8
Titan	72-77	6-48	310	0.2
	72-77	8-48	321	0.2
	72-77	10-48	328	0.2
	54-60	6-48	383	2.4
	54-60	8-48	368	3.7
	54-60	10-48	398	3.6
	Control		385	11.5
Newal	72-77	6-48	295	0.2
	72-77	8-48	276	0
	72-77	10-48	251	0
	54-60	6-48	348	5.3
	54-60	8-48	341	3.5
	54-60	10-48	370	4.0
	Control		346	9.4

the differences for period of immersion were not significant. In evaluating the best water treatment schedule from the standpoint of the effect in both varieties on smut control, stand and emergence it appears that treatment in standing water for a period of 56-64 hours at the temperature range indicated is the most satisfactory.

LOOSE SMUT OF WHEAT.—The effect of water soak and of Spergon treatment on the control of loose smut (*Ustilago tritici* ((Pers.)) of wheat was studied. The variety Stewart, used in these experiments, was treated in various ways, namely: seed immersed in 0.1 per cent Spergon suspension at room temperature and at 61° F. for 24, 28, or 32 hours, with and without a preliminary presoak for 6 hours in water; seed immersed in water for 38, 48, or 56 hours at room temperature, and at 61° F.; controls of dry seed treated with Ceresan M dust. The seed was planted in mid-May in 4 randomized 12-ft. rows, 200 seeds per row.

Wheat was injured more than barley by both the Spergon and water treatments. At room temperature (72–77° F.) Spergon eliminated loose smut, but at the price of severe reduction in germination of the grain. For example, the average number of heads per row was 83, 15, and 7, respectively, following treatment in 0.1 per cent Spergon for 24, 28, or 32 hours, preceded by 6 hours in water, as compared to 411 heads in the rows of untreated grain. At 61° F. similar treatments resulted in no significant reduction in stand but there were 0.6, 0.9, and 0.4 per cent smutted heads per row, respectively. The incidence of smut in control rows was 3.1 per cent.

The water treatment at room temperature resulted in almost as good control of smut as did the Spergon treatment. The 38, 48, or 56-hour periods of immersion gave 0.1 per cent smutted heads for the 38-hour period and none for the others. The stand was 363, 179, and 116 heads per row. At 61° F. the stand was apparently unaffected and the incidence of smutted heads was 1.1, 0.5, and 0.2 per cent, respectively, for the 3 periods mentioned above.

DISCUSSION.—Loose smut of barley may be as effectively controlled by soaking the seed in water at room temperature for 56 to 64 hours as by soaking it in 0.2 per cent Spergon suspension for 40 hours,

TABLE 3.—*Control of loose smut of barley by immersion in water at room temperature (72°–77° F.)*

Variety	Treatment	Immersion	Total heads	Smutted heads
		Hr.	Ave.	Per cent
Gem	Standing water	48	284	0.7
		56	236	0
		64	252	0.1
		72	100	0
		80	31	0
Gem	Water changed twice daily	48	294	0.1
		56	266	0
		64	228	0
		72	245	0
		80	163	0
Gem	Running water	48	295	1.7
		56	230	1.1
		64	241	1.4
		72	167	0.6
		80	179	2.2
Gem	Control		353	24.3
Warrior	Standing water	48	235	2.1
		56	259	0.5
		64	279	0
		72	259	0
		80	214	0
Warrior	Water changed twice daily	48	265	0.6
		56	284	0.1
		64	290	0.3
		72	265	0
		80	255	0
Warrior	Control		348	21.7

Analysis of variance (sine angle transformation) for Gem

Source	*D.F.*	*M.Sq*	*F*	$d_{.01}$	$d_{.05}$
Immersion	4	16.99	2.516	±2.89	±1.97
Treatment	2	146.27	21.65×	±3.07	±2.23
I. × T.	8	7.81	1.15		
Error	45	6.75			

preceded by 6 or 8 hours soak in water, or in 0.2 per cent Spergon for 48 hours with no presoak period in water. It was evident that temperature played an important part in the success of the control by each method. A protracted period of soaking of the seed at room temperature without Spergon leads to the growth of a rich microflora and a consequent fermentation and foul odor. Cosgrove et al[5] reported finding up to 0.05 per cent methoxy-p-benzoquinone in the fermented germ of wheat. Many quinones are fungicidal, and if such compounds or their precursors also occur in the germ of barley it is possible that they may be of importance in the elimination of the mycelium of loose smut from infected embryos. In any case it seems probable that some fungicidal or fungistatic condition is established within the embryo by soaking the infected seed in water.

The function of Spergon is not clearly defined at present but it was shown that when it was present in the liquid used to treat the seed, the elimination of the smut was hastened. This chemical, at the concentration used, allowed the growth of bacteria, but it appeared that certain species were suppressed, since decomposition did not occur to the same degree as in the seed soaked in water. It was evident, especially in the experiment on wheat treatment, that Spergon does penetrate the embryo and may kill the germ if the treatment is too prolonged. Also, it has been found that a dilution of 1:25,000 of Spergon in potato-dextrose-agar completely suppresses the growth from *U. nuda* spores placed on the surface of the medium.

[5] Cosgrove, D. J., D. G. H. Daniels, E. N. Greer, J. B. Hutchinson, T. Moran, and J. K. Whitehead. 1952. Isolation of methoxy-para-benzoquinone from fermented wheat germ. Nature 169: 966.

SUMMARY

Loose smut in the seed of various barley varieties was satisfactorily controlled by the following treatments in a temperature range of approximately 72°–77° F.: seed soaked in water for 56–64 hours; seed soaked in water for 6, 8, or 10 hours, followed by 40, 44, and 48 hours in 0.2 per cent Spergon suspension; and seed soaked in 0.2 percent Spergon suspension for 48 hours with no presoak period in water.

In the 56–64 hour water-soak treatments an odorous decomposition occurred, which was not evident in the Spergon treatments.

Loose smut in Stewart wheat was eliminated by soaking it in water for 48 hours at the temperature range mentioned above, and by soaking it in 0.1 per cent Spergon suspension for 24 hours. Mortality of healthy seed was severe in the latter treatment.

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STEM AND ROOT TEMPERATURES [1]

Paul E. Waggoner and R. H. Shaw [2]

Pathogens that exist at or near the soil line and outside of the host for a portion of their life cycle are exposed to considerable changes of temperature. The temperature of an object lying on the surface of exposed soil can be more than 20 F° warmer during the day and 5° cooler at night than air temperature at 45 cm. above the soil (1). The observations reported here show that in contrast to this rigorous climate, the climate which a pathogen encounters once it enters the host is temperate. These observations agree with others that the inner portions of tree trunks (5), succulent plant stems (2), and even steel rails (4) are cooler than the soil line at the point where they emerge.

Temperatures were measured by inserting bright copper-constantan thermocouples made of 30 gauge wire into the plants and recording the resulting potential by means of a Brown Electronik recording potentiometer. The instrument included a device for recording in 80 seconds the potentials produced by 16 thermocouples.

Temperatures were observed in and near a corn plant growing on mineral soil (Webster type). Thermocouples were threaded into the stem at heights of 2, 1, and 0 cm. and into the root at depths of 1, 2, 4, and 8 cm. At a depth of 16 cm. the roots were too small for the insertion of the thermocouple and it was wrapped around a root. Thermocouples were supported 2 cm. from the plant at the same heights and depths. The soil was then watered and left for 2 days to permit it to return to the condition of the surrounding cultivated soil. The plant and soil were shaded from direct insolation.

Observations taken at 4 and 11 AM on a clear August day are in Figure 1. The shaded plant was 10° cooler at 11 AM and 3° warmer at 4 AM than a bright thermocouple lying on the adjacent soil line. The temperature difference between plant and environment disappeared at a depth of 16 cm.

The effect of a different soil, plant, and exposure can be seen by other observations also taken in August (Figure 2). A tomato plant 45 cm. tall and another 30 cm. tall were growing 90 cm. from each other on peat soil. The soil near the large plant and the stem below 15 cm. were shaded by foliage while the soil near the small plant and its entire stem were exposed to direct insolation. Two thermocouples were laid on the soil line and 2 others were supported 1 cm. above upper exposed leaflets of both plants. A thermocouple was threaded into the stem of each plant at heights of 0, 10, and 20 cm. and into the growing points. Observations were taken in full sunlight at 11 AM.

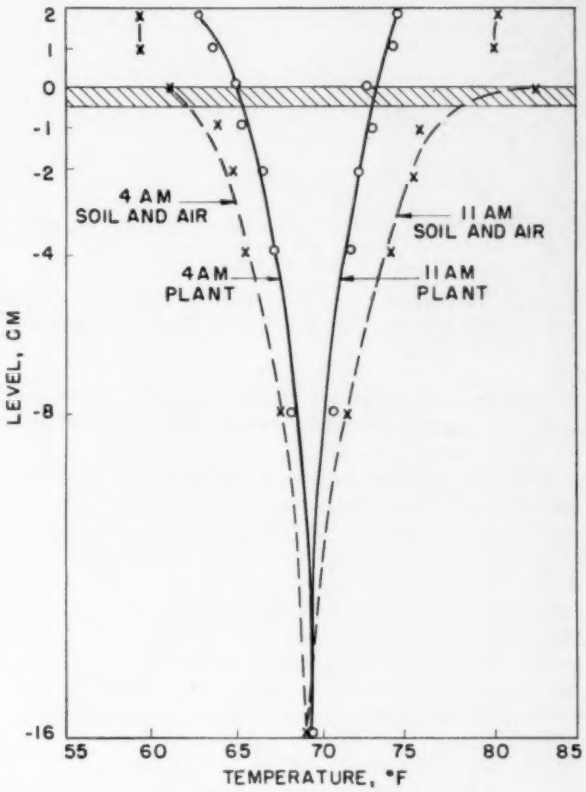

FIG. 1. Temperatures in and 2 cm. from the stem and root of a corn plant growing on Webster soil. August, sun unobscured.

The thermocouple lying on the exposed soil line near the small plant was 18° warmer than the one in the stem at the same height. The internal temperature of the stem at the soil line was within a degree of the air temperature at the top of the plant. The difference between the temperature of the thermocouple lying on the shaded soil near the large plant and the temperature in the stem at the same height was 11°, about the same as the difference between the temperature of the soil surface and the corn plant. The shaded tomato stem was 32° cooler than the thermocouples lying on the exposed soil. This stem was as much as 12° cooler than the air temperature at the top of the plant. Unlike the air, which is warmest at the soil, the stems are warmest at a height above the soil line. This agrees with the work of others (1,4). These observations in tomatoes growing on peat soil bear out the observations

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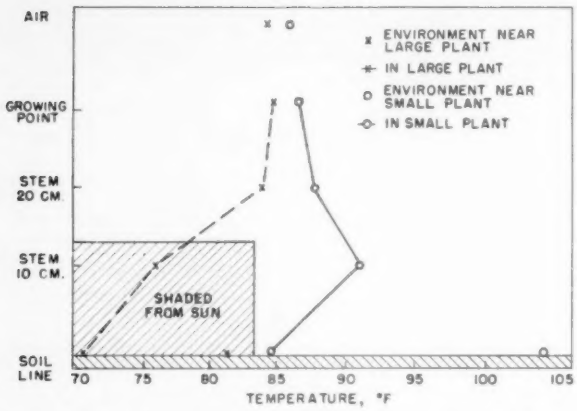

FIG. 2. Temperatures in and near the stems of a large and a small tomato plant growing on peat soil. August, sun unobscured.

in corn growing on mineral soil; temperatures in the stems during the day were cooler than temperatures on the adjacent soil line.

When a pathogen invades a host at the soil line it encounters a more temperate environment than the one it left, i.e., the soil surface. The environment is also tempered to a lesser degree at depths as low as 8 cm. and at heights up to about 5 cm. This observation is important in understanding the incidence of damping-off. Leach (3) has shown that damping-off is most severe at temperatures where the growth of the pathogen relative to the host is most rapid. In the cases of damping-off of spinach, peas, and sugar beets by *Pythium ultimum*, damage is a maximum at intermediate temperatures, 54°–77°, and is less at lower or higher temperatures. Now let us consider Leach's observations together with the observations of the preceding paragraphs that the temperature of stems and roots fluctuate less violently than the air or soil line temperatures. The moderation of temperature may be smaller in smaller stems, but it would nevertheless exist. We conclude that the temperature of the host can be more favorable for occurrence of damping-off than indicated by either air or soil surface temperature. Soil temperature at a depth of about 2 cm. should provide a better indication of likelihood of damping-off than soil surface or air temperature.

This study bears upon another problem in plant pathology. The observation that a shaded stem was 12° cooler than the air at the top of the plant suggests how a pathogen that is killed by high temperatures survives in a stem lesion through a period of hot weather. For example, *Phytophthora infestans* appeared on a large number of leaves in an Iowa potato field in 1950. The disease later disappeared except for a single stem lesion during a period of hot weather. When the weather again became favorable for late blight development, the fungus, which had probably survived in the cool stems, spread throughout the field (6). Thus, hot weather may not eradicate a fungus because it can be living in an environment whose maximum temperature is 12° less than that of the air.

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EFFECT OF LYCOMARASMIN DECOMPOSITION UPON ESTIMATES OF ITS PRODUCTION [1]

A. E. Dimond and Paul E. Waggoner

Lycomarasmin, produced in culture filtrates of *Fusarium oxysporum* f. *lycopersici*, is toxic to tomato cuttings and has been suggested as a vivotoxin in Fusarium wilt of tomatoes (3). Initially isolated and partially characterized by Plattner and Clauson-Kaas (5), lycomarasmin has been shown by Woolley (8) to be a dipeptide of asparagine and a new amino acid, N-(α-(α-glycyl) hydroxypropionic acid. Lycomarasmin forms a colorless complex with ferric ion and its toxicity to plants is potentiated by iron complex formation (5). This complex has been shown to be chelate in nature and to occur with either ferrous or ferric ions (7).

In the diseased plant foliar symptoms of Fusarium wilt appear within 15 days of inoculation. The fungus in the plant must produce at least the minimum toxic dose of lycomarasmin within this time. As lycomarasmin has not been isolated from diseased plants, the rate of production *in vitro* must be used to estimate lycomarasmin production *in vivo*. If the toxin decomposes rapidly at room temperature, the rate of production *in vitro* must actually be more rapid than the yield divided by the age of the culture. Consequently, under the assumption that the toxin in the plant acts soon after production and that the effect is permanent, the more rapid *in vitro* production rate should be used for estimating production in the plant. Experimentally determined decomposition rates are given in this paper and used to estimate the rate of lycomarasmin production *in vitro*. This rate in turn is used to estimate lycomarasmin production in the infected plant.

On heating in aqueous solution lycomarasmin decomposes to substance I (5), liberating ammonia from the amide nitrogen of the asparagine moiety (8), and simultaneously losing its biological activity (5). If lycomarasmin is unstable at elevated temperatures, it should also have a measurable rate of decomposition at temperatures encountered in culture flasks and diseased plants. This rate of breakdown at 25–30°C. has a bearing upon calculations of its rate of production in flasks and plants. Information on the effects of hydrogen ion concentration and complex formation upon rate of breakdown is essential to these calculations.

MATERIALS AND METHODS.—Thermal decomposition studies were based on the fact that for each initial mole of lycomarasmin, 1 mole of substance I and 1 mole of ammonia are produced as breakdown occurs. When the pH of the solution is below 7, ammonia will be trapped as ammonium ion. The ammonium ion liberated in aqueous solutions of lycomarasmin was removed by permutit, then liberated in alkaline solution, and estimated as ammonia nitrogen using Nessler's reagent (4). Colorimetric observations were made with a Fisher electrophotometer employing a 425 mμ filter.

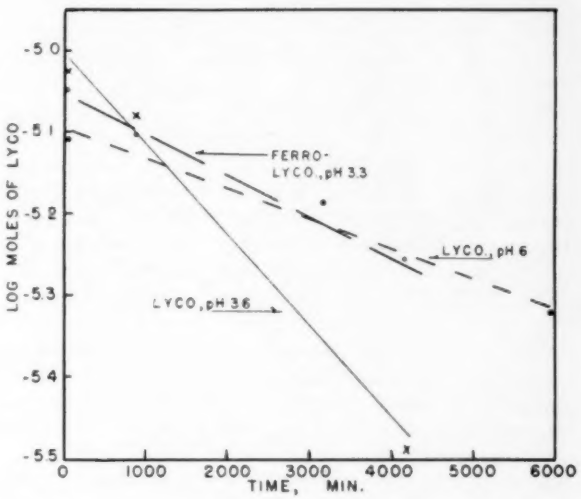

FIG. 1. Decomposition of lycomarasmin and ferro-lycomarasmin in aqueous solution at 28.5°.

A sample of pure lycomarasmin (m.p. 228°), kindly furnished by Dr. E. Gaumann, was used. The rate of decomposition of lycomarasmin in water, pH 3.6, and in .058 M phosphate buffer, pH 6, was measured. 10^{-5} mole of lycomarasmin was initially present in each sample analyzed. The rate of decomposition of ferro-lycomarasmin in water, pH 3.3, was measured. Buffer was not used because it would interfere with the iron. 10^{-5} mole of lycomarasmin and 0.5×10^{-5} mole of ferrous sulfate was initially present in the ferro-lycomarasmin samples.

RESULTS.—The course of lycomarasmin and ferro-lycomarasmin decomposition at 28.5° is shown in Figure 1. As anticipated, decomposition is appreciable even at temperatures encountered in culture flasks and diseased plants. This partially accounts for the observation (3) that the minimum toxic dose is apparently a function of the concentration absorbed by cuttings; when a dilute solution must be absorbed for a long time, decomposition in the solution further increases the time necessary for a toxic dose to be accumulated.

Decomposition at 28.5°, 45°, and 63° is described by the half-lives in Table 1. From these data the half-lives at other temperatures and energies of activation of the decomposition reaction can be calculated by the usual methods of thermodynamics (1).

Lycomarasmin is more stable at pH 6 and pH 3.6 than at the temperature of Table 1, but would be equally stable at either pH at 0° C. Thus, the lower heat of activation at pH 6.0, 16 K cal., compared to the higher

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TABLE 1.—*Half-lives of lycomarasmin and ferro-lycomarasmin*

Temperature °C.	Lycomarasmin pH 6.0	Lycomarasmin pH 3.6	Ferro-lycomarasmin pH 3.3
28.5°	5.9 days	1.8 days	4.2 days
45	28.6 hours	9.4 hours	13.2 hours
63	560 min.	65 min.	99 min.
Heat of activation K cal.	16	22	25

one at pH 3.6, 22 K cal., does not entirely predict the 2 sets of half-lives. This indicates that the entropy of activation is a function of hydrogen ion concentration. The inequality is not surprising if decomposition involves rearrangement of the molecule as suggested by Woolley (8).

The heats of activation of lycomarasmin (pH 3.6) and of ferro-lycomarasmin (pH 3.3) decomposition would be predicted to differ on physical grounds. A small part of the 3000 calorie difference may have resulted from the effect of pH. The major part of this difference, however, is probably related to the greater number of bonds that have been broken. The position of the chelate linkage in lycomarasmin determines this stabilizing effect.

Additional experimental evidence is necessary to determine the position of the chelate linkages in lycomarasmin. Lycomarasmin forms colorless chelate complexes with iron (7). 8-Quinolinol forms colored chelate complexes with iron ions. The stability of the quinolinol-chelate complex is greater than that of the lycomarasmin complex at pH 6 (7), but decreases at lower hydrogen ion concentrations. Therefore, if 8-quinolinol is added to mixtures containing varying ratios of lycomarasmin and iron, and a short development time is used, it should be possible to determine colorimetrically the amount of free iron not combined with lycomarasmin. Thus, in 1 experiment (Table 2) lycomarasmin and ferrous sulfate were added in varying stoichiometric proportions so that the final concentration of ferrous sulfate was always 10^{-3} M. 8-Quinolinol was then added to bring its concentration finally to 2×10^{-3} M, and after 10 minutes the depth of color was read through a 425 mμ filter. The pH of these solutions varied from 3.3 to 3.6. As the quantity of lycomarasmin and the quantity of iron ion bound by lycomarasmin increases, the amount of free ferrous ion indicated by the method decreases. Therefore, the method was employed to see if chelating ability is lost

TABLE 2.—*Relative free ferrous ion concentration in lycomarasmin solutions*

Lycomarasmin	Relative free ferrous ion	Toxicity to cuttings
0 M	100%	None
10^{-3}	43	Moderate
2×10^{-3}	1	Severe

TABLE 3.—*Relative amount of free ferrous ion in lycomarasmin solutions*

Lycomarasmin	Free ferrous ion	Toxicity to cuttings
0	100%	None
10^{-3} M	17	Severe
10^{-3} M heated for 45 min. at 24–86°	50	Moderate

as lycomarasmin decomposes to substance 1.

The experiment summarized in Table 3 shows that chelating ability is lost. This suggests that the amide nitrogen lost by decomposition participates in the chelate bond. Therefore, more energy is required to activate the decomposition of the complex than of lycomarasmin. That is, the necessity of breaking the more stable chelate bond explains the higher activation energy of ferro-lycomarasmin as compared to lycomarasmin, Table 1.

A second inference can be drawn from Table 3. Because a correlation exists between chelating ability and toxicity, lycomarasmin probably acts as ferro-lycomarasmin. This supports other evidence which shows that copper ion inhibits lycomarasmin toxicity by preventing the formation of ferro-lycomarasmin (7).

DISCUSSION.—From the studies of decomposition and from a statement of *in vitro* yield of lycomarasmin, .06 mg./ml. in 60 days (5), the production rate of lycomarasmin can be inferred. In a quiet culture of *Fusarium* in a modified Richard's medium at room temperature, mycelium reaches a maximum weight in about 30 days and lycomarasmin first appears about 40 days after inoculation (2). Let us assume that the lycomarasmin production per day per initial ml. of medium is constant from 30–60 days. Then

$$dc/dt = -kc + p$$

where c is lycomarasmin concentration per initial ml. of medium, k is specific decomposition rate, and p is production rate. If lycomarasmin concentration is 0 at 30 days and .06 mg./ml. at 60 days,

$$\ln \{p/(p-.06)\} = 30k.$$

At 28.5°C. k was found to be .37 and .11 per day for lycomarasmin at pH 3.6 and 6.0 and .17 per day for ferro-lycomarasmin at pH 3.3. Substituting these in the preceding equation the production rate, p, is found to be .019 or .007 mg./ml. per day if k for lycomarasmin at pH 3.6 or 6.0 is used. Production is found to be .010 mg./ml. per day if k for ferro-lycomarasmin is used. Because the half-lives are much shorter than 30 days, the estimates of production rates are not changed significantly if lycomarasmin concentration is set at 0 for day 0 rather than day 30.

The decomposition rate of .007 mg./ml. per day based on the case of lycomarasmin at pH 6 seems to represent best the situation in culture media. This decision is based upon the fact that the lycomarasmin in culture filtrates is not complexed with iron but can be potentiated by the addition of iron and upon the fact that the pH in cultures is greater than 6 when lycomarasmin appears (2).

Let us now see how the estimate of *in vivo* production of lycomarasmin is affected by using these rates. Scheffer (6) has estimated that a tomato plant a foot high has a void volume of 0.1 ml. and requires 0.25 mg. of lycomarasmin to show symptoms. We make the assumption that this void volume contains as much mycelium per unit volume throughout the incubation period as the culture flask does in the second month. We further assume that the rate of lycomarasmin production by the rapidly growing *Fusarium in vivo* is the same as by the mycelium *in vitro* while it is losing weight. These 2 assumptions lead to an estimated production of .01 mg. per plant in 15 days, 1/25 of the minimum toxic dose, if the production rate chosen above is used. If the highest production rate, 0.22 mg./ml. per day based on lycomarasmin at pH 3.6, is used, the estimated production per plant in 15 days is raised to .033 mg. Even this is only about 1/8 of the minimum toxic dose.

The assumptions underlying the foregoing estimates have favored high lycomarasmin production: the void volume of the plant is assumed to contain as much lycomarasmin per ml. from inoculation time to 15 days as a 30 day old culture. Further, the rapidly growing mycelium is assumed to produce as much mycelium as 30–60 day old cultures. (Studies of the course of *Fusarium* growth and lycomarasmin production *in vitro* suggest that 30–60 day old cultures have a higher production rate than younger ones (2)). Therefore, even after adjusting production rates for decomposition *in vitro* we cannot say that sufficient lycomarasmin is produced *in vivo* to produce Fusarium wilt symptoms.

SUMMARY

Lycomarasmin, a toxin isolated from culture filtrates of *Fusarium oxysporum* f. *lycopersici*, is known to decompose to the non-toxic substance I with the liberation of ammonia. At 28.5°C. lycomarasmin has a half-life of 5.9 days at pH 6.0 and of 1.8 days at pH 3.6. At the same temperature ferro-lycomarasmin has a half-life of 4.2 days. The greater stability of lycomarasmin at room temperature and pH 6.0 than at the same temperature and pH 3.6, while the heat of activation of the decomposition at pH 6.0 is the lower of the 2, indicates that the entropies of activation are not equal at the 2 hydrogen ion concentrations. Because the amide nitrogen lost in the decomposition participates in the chelate bond and because of a lower pH, the heat of activation of ferro-lycomarasmin at pH 3.3 is less than that for lycomarasmin decomposition at pH 3.6. Using the decomposition rates found and assuming that lycomarasmin production rate *in vitro* is constant after the mycelium reaches maximum weight, the production rate is estimated from published yields. Employing the resulting rates, we cannot say that sufficient lycomarasmin is produced *in vivo* to produce Fusarium wilt symptoms.

As lycomarasmin decomposes to substance I it loses both iron-chelating ability and wilt inducing ability. Lycomarasmin chelates other metals but these are not toxic to plants. These facts suggest that lycomarasmin is active on plant cells as the iron-lycomarasmin chelate complex.

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FUSION BETWEEN SECONDARY SPORIDIA IN CULTURE AS AN INDEX OF SEX COMPATIBILITY IN TILLETIA SPECIES [1]

C. S. Holton [2]

The determination of sex compatibility factors is essential to adequate investigations of the biology of the smut fungi. In *Tilletia* species such determinations have been based on fusion between primary sporidia (3) or on infection results from inoculation with paired monosporidial lines (1, 2, 3, 4). Both these methods have definite limitations and disadvantages which were discussed in a previous paper (4). At that time the need was emphasized for a method of determining compatibility relationships between monosporidial lines in culture. Recently such a method has been developed, if fusion between secondary sporidia in culture may be accepted as an index of sex compatibility.

SPORIDIAL FUSION.—Monosporidial lines derived from single primary sporidia of *Tilletia caries* (DC.) Tul. were used. These were maintained in stock culture on Difco potato-dextrose agar. Matings between sporidia were made on a suitable medium by placing them adjacent to each other with the aid of a micromanipulator. Compatible pairs fused within 1 to 3 hours by means of a fusion tube similar to that which joins fused primary sporidia. Each fused pair of sporidia produced a single hypha which would not grow in culture, thus indicating dikaryosis, the obligately parasitic stage. In contrast, nonfused (incompatible) pairs of sporidia developed culture colonies. Fusion also occurred between primary and secondary sporidia. Development of these fused pairs was generally the same as that of fused secondary sporidia.

FACTORS INFLUENCING SPORIDIAL FUSION.—The percentage of fusions between compatible pairs of sporidia was influenced by promiximity and by the kind of medium. Cursory observations indicated that fusion occurred more readily between sporidia lying close together. To test the influence of proximity on fusion, 30 pairs of sporidia, representing 2 compatible monosporidial lines, were divided into 3 groups of 10 pairs each. In 1 group the sporidia of each pair were placed in direct contact; in the second group the sporidia were separated by approximately 10μ; and in the third group they were placed approximately 50μ apart. The percentages of fused pairs in the 3 groups were 100, 70, and 30, respectively. While the numbers involved in this test were small, the results confirm the observations made previously on many pairs of sporidia that proximity is an important factor in fusion between secondary sporidia of *Tilletia* spp.

Three kinds of media were used to test influences on sporidial fusion. These were water agar (2 per cent), potato-dextrose agar, and Zscheile's (6) T-1 medium. Several tests were made by placing compatible pairs of secondary sporidia on each kind of medium. On water agar, of 50 pairs, only 5 (10 per cent) fused; on potato-dextrose agar 54 of 68 pairs (79.4 per cent) fused; and on Zscheile's T-1 medium 51 of 55 pairs (92.7 per cent) fused. Obviously a nutrient medium stimulates the fusion process, and there is some indication that Zscheile's T-1 medium is better than potato-dextrose agar. Similar results were obtained when pairings were made between primary and secondary sporidia.

COMPATIBILITY FACTORS.—The infective capacity of paired monosporidial lines has long been the principal index of sex compatibility in *Tilletia* spp. Flor (2) obtained evidence of multiple sex-compatibility groups by this method. In contrast, only 2 compatibility groups were indicated in the results reported by Hanna (3), Becker (1), and Holton (4). Becker (1) also obtained evidence of a more complex relationship in pairings between monosporidial lines from different spores. Only simple relationships were indicated in the matings between secondary sporidia reported here.

Matings were made between secondary sporidia representing monosporidial lines from the same as well as from different spores. All possible combinations were made among each set of monosporidial lines, and 2 of the sets were mated in all possible cross combinations. The pattern of reaction between paired sporidia, as indicated by fusion, is illustrated in Table 1 and clearly shows a simple 2-factor grouping. There was also evidence of a sex-linked lethal deficiency comparable with that reported previously (4). Only one-half the primary sporidia of 4 spores (Nos. 265, 266, 267, 268) produced culture colonies (Table 1). Three of these

TABLE 1. *Compatibility grouping in 9 sets of monosporidial lines of Tilletia caries, as determined by fusion between pairs of secondary sporidia in culture*

Spore Number	Number of Primary Sporidia Produced	Number of Monosporidial Lines Obtained	Compatibility Groups Plus (+)	Compatibility Groups Minus (−)
256	8	7	2,3,4	1,5,6,7
258	8	8	3,4,6,8	1,2,5,7
259	18	12	1,2,5,6,8,11,12	3,4,7,9,10
263	11	11	1,4,9,10,11	2,3,5,6,7,8
255	10	8	1,5,8	2,3,4,6,7
265	8	4	1,2,3,4	----
266	8	4	1,2,3,4	----
267	8	4	----	1,2,3,4
268	8	4	1,2,3,4	----

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TABLE 2. *Compatibility relationships in Tilletia caries as indicated by fusion between primary and secondary sporidia*

Source of Primary Sporidia	Source of Secondary Sporidia [a]	Number of Pairs Made	Pairs Fused	
			No.	Per cent
Dwarf Col. 7	259-8	53	24	47.0
	259-10	144	135	93.7
Dwarf Col. 3	259-8	66	28	12.4
	259-10	174	139	79.8
Dwarf Col. 10	259-8	34	4	11.8
	259-10	52	42	80.7
T-9	259-8	21	4	19.0
	259-10	45	13	28.4
Hybrid 136 [b]	259-10	51	5	9.8

[a] Monosporidial lines 8 and 10 from chlamydospore 259 of race T-9.
[b] Dwarf Col. 10 × Race T-10.

sets were of the same sex-compatibility group, while the fourth (No. 267) was of the opposite compatibility group. Thus the lethal deficiency was sex-linked but not always with the same sex factor. The results presented in Table 1 were confirmed by all matings between secondary sporidia, whether from the same or from different spores. For example, the plus (+) and minus (−) lines of spore 256 were compatible with the minus (−) and plus (+) lines respectively of spore 258. Similarly, the plus (+) and minus (−) lines of spore 263 were compatible with all lines of opposite designation from spores 265, 266, 267 and 268 (Table 1). In matings between secondary and primary sporidia, however, a different kind of relationship was indicated.

In these matings, single secondary sporidia from 2 monosporidial lines of the opposite sex were paired in sets with all the primary sporidia from 42 germinating spores. The germinating spores represented 3 collections of dwarf bunt, race T-9 of common bunt, and a hybrid (No. 136) between dwarf and common bunt. The number of matings for each set was equal to the number of primary sporidia produced by each spore, ranging from 7 to 22. Unique compatibility relationships were exhibited, as shown in Table 2.

Based on percentage of fusing pairs, the primary sporidia expressed a wider compatibility relationship with line 259-10 than with line 259-8 (Table 2). Furthermore, though not shown in Table 2, all the mated pairs in 7 sets involving line 259-10 fused, while none of the sets involving line 259-8 produced 100 per cent fusions. Actually, usually less than half the pairs fused where line 259-8 was involved. Thus, whereas lines 259-8 and 259-10 are of opposite sex in relation to each other and to other monosporidial lines, they are not completely opposite in relation to primary sporidia. It was apparent also that both lines were more compatible with the primary sporidia of dwarf bunt than with those of T-9, from which race they were derived. The lowest degree of compatibility was exhibited in matings with the primary sporidia of Hybrid 136 (Table 2).

Obviously these results do not conform to the compatibility patterns previously established by fusion between primary sporidia (3) and by pathogenicity tests (1,2,3,4). However, since these results were from matings between sporidia on agar media, they need to be confirmed by inoculation studies, which are necessary to establish the validity of sporidial fusion as a compatibility index.

SUMMARY

Secondary sporidia of *Tilletia caries* fused in culture when placed close together on a suitable medium. Placement of the sporidia in pairs was accomplished with the aid of a micromanipulator. The sporidia fused more readily when lying next to each other than when farther apart. Fusion also occurs more readily on a nutrient medium such as potato-dextrose agar or Zscheile's (6) T-1 medium than on water agar.

Compatibility relationships, as indicated by fusion between secondary sporidia, were on a simple 2-factor basis. This was true in matings between sporidia from the same or different spores. Unique relationships were indicated in matings between secondary and primary sporidia. Monosporidial lines of opposite sex, as indicated by fusion between secondary sporidia, were not completely opposite in their relationships with primary sporidia, as indicated by fusion between primary and secondary sporidia. These results need clarification by inoculation experiments.

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STUDIES ON STONE-FRUIT VIRUSES IN CUCURBIT HOSTS. II. SOME FACTORS AFFECTING THE AGING OF INOCULUM IN VITRO [1]

R. S. Willison and M. Weintraub

Varney and Moore[2] have shown that 2 isolates from sour cherry varied differentially in the symptoms they induced on cucumber plants grown at various temperatures between 61° and 95°F. Willison[3] reported differences in the reaction of cucumber to infection with several isolates from cherry and plum. He also found[3, 4] that symptom expression varied with differences in the length of the daily period of illumination at constant temperature. Boyle, Moore, and Keitt[5] reported that the virus transmitted to cucumber from sour cherry showing symptoms of necrotic ringspot had a thermal inactivation point of 52°C. for 10 minutes and a dilution end point of 1/20. Expressed juice remained infectious for only 12 hours at room temperature. Very little else is known about the characteristics of the stone-fruit viruses themselves.

In investigating the nature and properties of those stone-fruit virus entities that could be mechanically transmitted to cucurbit hosts, it soon became apparent that the various isolates were relatively and variably unstable in expressed sap. Consequently, little progress could be made until information was obtained concerning the conditions and factors affecting the infectivity and longevity of the virus isolates *in vitro*. The investigation of this phase of the problem is discussed in the present paper.

MATERIALS AND METHODS.—The isolates used in these experiments were obtained from sweet and sour cherries and plums affected with different diseases. The isolates are grouped according to the symptom type observed on the *Prunus* hosts. Each group is designated by a letter as follows: D, prune dwarf; G, green ring mottle; N, necrotic ringspot; R, a recurrent chlorotic ring pattern on sour cherry; T, tatter leaf of sweet cherry; and Y, cherry yellows. The figure appended to each letter serves to distinguish between isolates within each group. For example, isolates N.1, N.2, and N.3 came from 3 trees, 1 Lombard plum and 2 Montmorency cherries, affected with latent necrotic ringspot.

The sources of the inocula with which mechanical transfers were made included young peach leaves, and symptom-bearing leaves of cucumber, vegetable marrow, and pumpkin. References to age of source of inoculum indicate the length of time that the source plants were infected before being used for inoculum. Assays of infectivity of inoculum were made on uniform seedlings of the cucumber variety Windermoor Wonder. The number of plants used for each assay was usually 6, or sometimes 10.

Inoculum was prepared by grinding infected leaves in a mortar to which a small quantity of distilled water had been added. Distilled water was then added to the mash to bring the volume up to 4 or 10 ml. for each gram of leaf (dilutions equivalent to 2^{-2} and 10^{-1}). Higher dilutions were made from the 10^{-1} dilution in the usual way. Experiments were conducted with crude juice. Straining through broadcloth usually resulted in reduction or loss of infectivity. No consistent differences in infectivity were observed between crude juice and the supernatant liquid from which the coarse debris had been centrifuged at approximately $3000 \times G$.

The cucurbit hosts were inoculated when the first true leaf was starting to develop. One cotyledon of each plant was lightly dusted with abrasive powder and inoculum was applied by rubbing, usually with 12 strokes of the finger. The inoculating technique was standardized as much as possible, although minor variations had no appreciable effect on results.

Progressive changes in the infectivity of an inoculum were evaluated by means of a 5-point infection rating scale, based on the severity of induced symptoms and the proportion of assay plants infected (4). Infection ratings on each set of assay plants were read at 2 or 3 days intervals until no significant change was observed between 2 successive readings. This stabilized rating or end point was used in assessing infectivity throughout the experiments described below. Whenever direct comparisons are made, the ratings given were read on the same day.

EXPERIMENTAL RESULTS.—Limitations of space prevent the presentation of all the experimental data accumulated on the phase of the problem under discussion. Results reported are, however, representative, having been obtained either under comparable circumstances or, with some variation in detail, on more than one occasion.

Effect of temperature of storage of inoculum.—Aliquots of inoculum of isolate Y.3, prepared from young peach leaves were held at room temperature, and at 0°C. in a bath of ice-water. Assays were made immediately after maceration and 1, 2, 4, 8 and 12 hours later. The inoculum was not infective after 1 hour at

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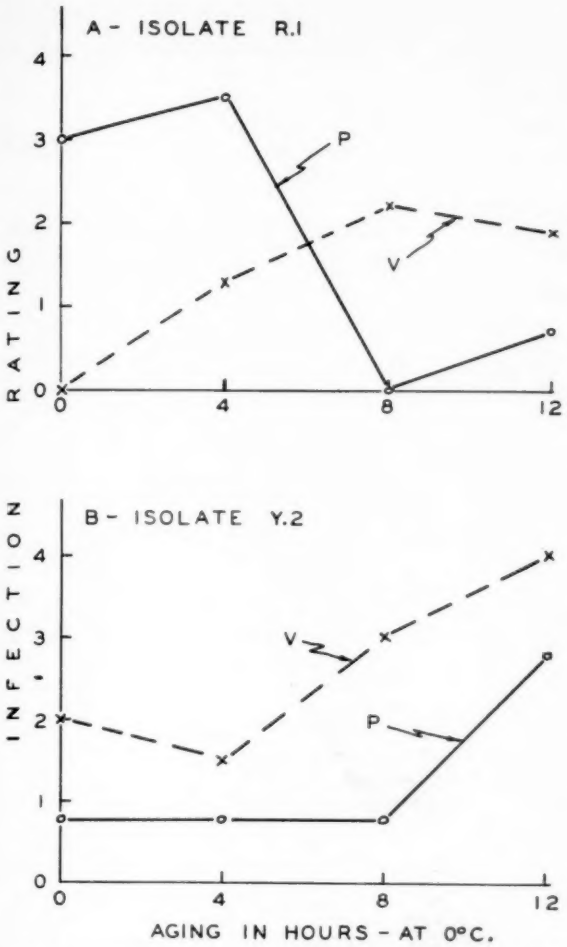

FIG. 1. Effect of aging on infectivity of isolates R.1 and Y.2 in inoculum prepared from vegetable marrow (V) and pumpkin (P). Source plants infected 44 days before assay. Assays made simultaneously under the same conditions. Dilution 2^{-2}.

room temperature and at 0°C. showed progressive loss of infectivity at successive intervals, becoming non-infectious at 12 hours. In a similar experiment with isolate N.1, inoculum prepared from cucumber sources lost its infectivity in 4 hours at room temperature, but remained infective for more than 24 hours at 0°C. (Table 1). The behavior of isolate T.2 under similar circumstances is shown in Fig. 3. Variations observed in the longevity of any 1 isolate either at room temperature or at 0°C. indicate that factors other than holding temperature are also operative.

Effect of source of inoculum.—The longevity of isolate Y.3 in inoculum from peach has already been indicated as 12 hours or less at 0°C. The same virus entity in inoculum from cucumber showed little loss of infectivity over a 24-hour period at 0°C.

Systemic infection with stone-fruit virus isolates proceeded much more slowly and was less severe in vegetable marrow and pumpkin than in cucumber. In 1 experiment with isolates R.1 and Y.2, infected vegetable marrow and pumpkin plants were available for assay 44 days after inoculation, long after comparable cucumber plants had been assayed and discarded. Inocula from these 44-day old sources were aged simultaneously at O°C. and assayed at 4-hour intervals. With isolate R.1, infectivity levels in the marrow and pumpkin inocula differed widely at successive time intervals (Fig. 1,A). In one case, infectivity or effective virus concentration rose and, in the other, dropped sharply over the same 12-hour period. These results can be attributed neither to differences in susceptibility of assay plants nor to differences in actual or potential virus concentration in the 2 inocula. As the same isolate was involved, the behaviour of the 2 inocula can be best explained by differences in the host-virus relationship. The curves obtained with isolate R.1 also differed in character from those obtained with isolate Y.2 under the same conditions of assay (Fig. 1,B). This isolate appears to have been at somewhat higher concentration in the vegetable marrow than in the pumpkin inoculum. The significant feature of this part of the experiment is the rise in the effective concentration of virus as both inocula aged.

Effect of age of source of inoculum.—Inocula of isolate N.1 were prepared at the same time from cucumber sources infected for 7 and 25 days. The preparations were aged at 0°C. and assayed under the same conditions. The inoculum from the older source became progressively less infective over a 12-hour period, while that from the younger source remained apparently unchanged (Fig. 2). The difference in the behaviour of the 2 inocula was undoubtedly due to differences in both potential and effective virus concentration, determined largely by factors associated with the respective ages of the sources. Other isolates, however, were not necessarily affected in the same way. For example, no appreciable difference in aging was observed with inocula of isolate G.1 from cucum-

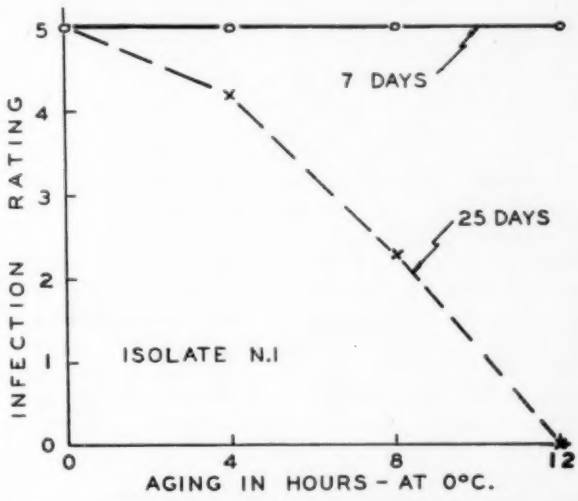

FIG. 2. Effect of age of source on aging of inoculum. Source plants infected with isolate N.1 for 7 and 25 days before assay. Assayed Sept. 18, 1951, under the same conditions. Dilution 2^{-2}.

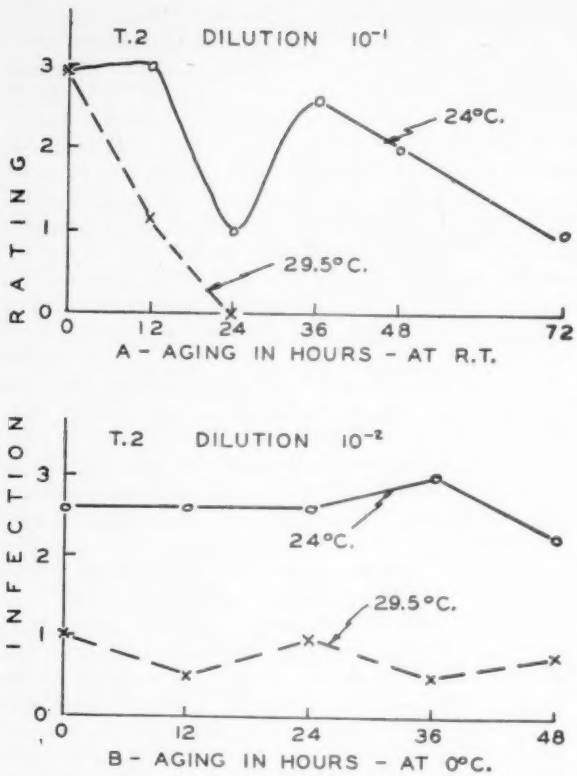

FIG. 3. Effect of growing source plants at 24°C. and at 29.5°C. on aging of inoculum (A) at room temperature and (B) at 0°C. Source plants infected with isolate T.2 for 9 days before assay. Assays A and B were made at different dilutions but at the same time and under the same conditions.

ber sources 9 and 30 days old, whereas isolate D.1 resisted the effects of aging longer in inoculum from a 28-day-old source than in that from a 13-day-old source. The effects of age of source of inoculum on the longevity of any of the isolates appeared to depend to some extent on the severity of the induced disease. When symptom expression on source plants was less severe, aging of inoculum was less affected by differences in the age of the sources.

Effect of conditions during the growth of source plants.—Eighty cucumber plants were inoculated with isolate T.2. Half the plants were then held at 24°C., and the other half at 29.5°C. Both sets of plants were illuminated 12 hours daily. After 9 days, systemic symptoms were somewhat less pronounced on the plants grown at the higher temperature. At that time, inocula at a dilution of 10^{-1} was prepared from each source. Aliquots of each inoculum were aged at room temperature and at 0°C. Additional aliquots were diluted to 10^{-2} for aging at 0°C. At room temperature inocula from sources grown at 24° and 29.5°C. remained infective for more than 72 hours and less than 24 hours respectively (Fig. 3,A). At 0°C., no appreciable changes in infectivity were noted over a 48-hour period in either inoculum at either dilution (Fig. 3,B). The difference in level between the curves for aging at 0°C. at a dilution of 10^{-2} indicate a lower level of both potential and effective virus concentration in the inoculum from the source grown at the higher temperature. Almost identical results were obtained in a parallel experiment conducted at the same time with isolate G.1. The temporary depression of infectivity in the more potent inoculum at 24 hours at room temperature (Fig. 3,A) also occurred with isolate G.1. As no such depression developed in the aging experiment at 0°C. (Fig. 3,B), this phenomenon cannot be attributed to diurnal fluctuations in the susceptibility of assay plants.

Effect of conditions during growth of assay plants.—It has already been shown (2,3,4) that the severity of the induced disease and the resultant possible maximum infection rating can vary considerably with the environmental conditions under which infected plants are grown. These variations can affect the position and slope of assay curves, but can have no direct influence on the effective concentration of virus in an inoculum. Thus, effects of treatments on an inoculum with respect to characteristics, such as thermal inactivation, longevity, etc., can be determined within the wide range of conditions under which systemic infection can take place. On the other hand, critical comparisons can be made only between assays carried on under similar conditions.

Effects of dilution.—Most of the isolates tested had a dilution end point slightly higher than 10^{-2}, whereas isolates G.1 and T.2 were infective at 10^{-4}. Obviously, longevity tests run at dilutions close to the end point may not be reliable. Dilution between 2^{-2} and 10^{-1}, however, usually appeared to have little effect on aging of inoculum. Within this range, the more dilute inocula, if anything, tended to be more resistant to the effects of aging than did the more concentrated.

TABLE 1.—*Time in hours for isolates to become noninfective in inocula assayed at different times and under different conditions*

Isolate	Hours to lose infectivity: Aging at R.T.	Aging at 0°C.	Time limit [c] (Hours)
D.1	<1	from <12, to >24	48
G.1 [a] A	–	from <12, to >36	48
G.1 B	>72	>96	96
G.2	–	<12 [b]	12
G.3	–	circa 48 [b]	48
N.1	circa 4 [b]	from <12, to >48	48
N.2	–	from <12, to circa 48	48
N.3	–	from <12, to circa 24	48
R.1	–	>12	12
R.2		from >12, to <24	24
T.2 [a] A	–	circa 48	48
T.2 B	>72	>96	96
T.3	–	from <24, to >36	48
Y.2	circa 12 [b]	from <12, to circa 48	48

[a] Isolates G.1 and T.2 each have at least 2 distinct separable components.
[b] 1 test only.
[c] Maximum aging period tested.

Comparison of isolates.—The influence of the above-mentioned factors on infectivity of inoculum makes it difficult to assess differences between isolates. This difficulty is well illustrated by the range of results obtained in a number of assays of individual isolates (Table 1). There were, however, indications that considerable differences in resistance to aging *in vitro* do exist between isolates, particularly those in the green ring mottle and tatter leaf groups. These groups will be treated more fully in a later paper in this series. Further evidence of differences between and within the several groups of isolates was obtained in a series of 3 experiments conducted over a 4-day period in September, 1951. Conditions of assay were essentially the same for the 3 experiments. In an aging period of 12 hours, no 2 inocula, with the exception of those of isolates N.1 (from the 7-day old source) and Y.3, reacted in the same way (Figs. 2 and 4). Neither differences in age of source of inoculum nor fluctuations and variations in the susceptibility of assay plants can account for all the differences in the behavior of the various isolates. Of particular importance are the abnormal curves obtained with isolates Y.2 and G.2, both of which show tendencies towards a recovery of infectivity *in vitro* after the passage of several hours (Fig. 1 and 4).

Effect of pre-inoculation conditions.—Although no formal experiments were carried out, observations during the winter of 1951–2 made it abundantly evident that preinoculation conditions could be a limiting factor in assays of infectivity. Until early November, no difficulty was encountered in maintaining the isolates in the greenhouse. Throughout the winter, cucumber plants were raised to inoculable age in the greenhouse. From November to April, all plants were placed immediately after inoculation in a controlled environment, for the most part at a constant temperature of 24 to 25°C. with artificial illumination for 12 hours daily. During November, it was possible to run tests with isolates G.1, T.2, N.1, N.2 and Y.2. During December and January, when the cucumber seedlings developed very slowly in the greenhouse, the last 3 isolates were maintained with difficulty, relatively few inoculated plants becoming infected. However, after early February, it again became possible to work with these isolates, although no changes were made in the controlled environment. On the other hand, isolates G.1 and T.2 remained highly infective throughout the winter. It is significant that the longer-lived components of these isolates (foot-note (a) Table 1) became predominant during that time. It would appear that cucumber seedlings became more resistant to infection with some isolates but not with others as conditions for germination and growth became more unfavorable.

Discussion.—The normal progress of aging of virus *in vitro* would be expected to be marked by either a gradual or a sudden decline in infectivity. Several examples of such normal aging have been presented above. The longevity of most of the stone-fruit virus isolates tested could be measured in hours rather than

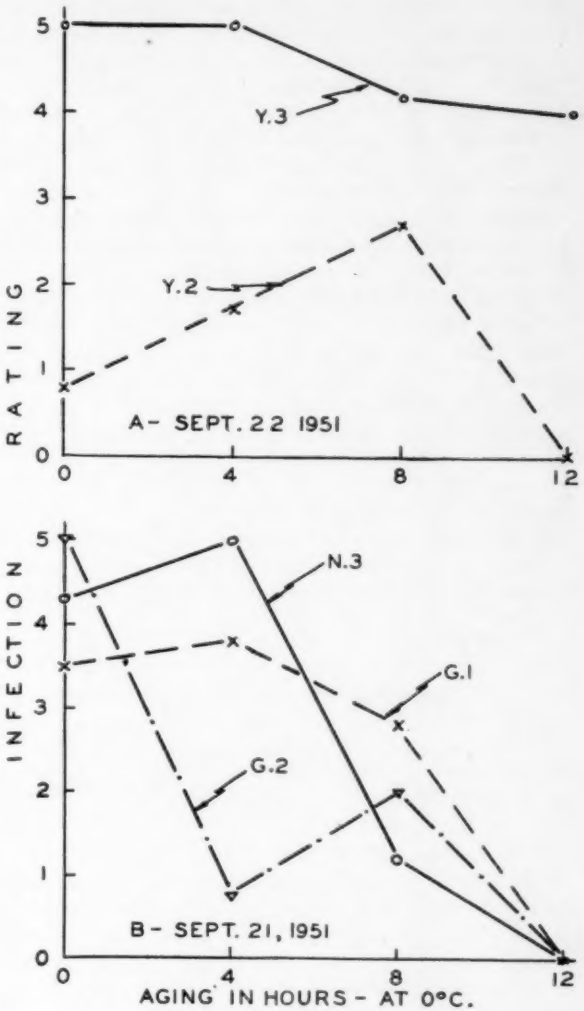

Fig. 4. Comparison of 5 different isolates in inocula aged at 0°C. and assayed under the same conditions. Source plants infected A, 15 days and B, 7 days before assay. Dilution 2^{-2}.

in days. Nevertheless there was evidence that the end-point of normal aging depends, not only on virus concentration and on the isolate used, but also on the species and age of the sources of inoculum. In addition, there have been numerous instances of infectivity increasing with aging. Such increases may or may not have been preceded by a decline. Since, in parallel tests carried on at the same time and under the same environmental conditions, aging has proceeded normally with some isolates and abnormally with others, the abnormal results could scarcely have been due to changes in susceptibility of assay plants. It would therefore appear that longevity and aging can also depend on the interaction of virus entities with other constituents of the inoculum. Increases in the infectivity of inoculum over a period of time suggest 1) that the actual or potential virus concentration was

in some and probably in all cases considerably higher than the effective virus concentration which gave rise to infection and 2) that additional virus could be released to raise the effective virus concentration. The release of virus in the course of a few hours implies a temporary and reversible inhibition of a least part of the virus by some constituents of the cell sap or debris. The inhibiting factors appear to vary considerably in inocula from sources of either different age or different kind and in their effect on different isolates. This phase of the investigation will be discussed in greater detail in the next paper of the series.

It has been shown that the various stone-fruit isolates differ from one another, not only in the type and severity of symptom expression on cucurbit hosts (3), but also in their longevity and behaviour during the aging process *in vitro*. Some of the differences, such as those between components of isolates G.1 and T.2 (Table 1), are wider than those that would be expected to occur between strains of the same virus. The full significance of the differences between other isolates must remain in abeyance until further information is available. On the basis of the data so far presented, it is not possible to determine whether isolates associated with the different symptom groups are related strains or not.

SUMMARY

Isolates of stone-fruit virus entities have been transmitted to cucurbit hosts from trees affected with prune dwarf, green ring mottle, necrotic ringspot, a recurrent ring pattern, tatter leaf and cherry yellows. These isolates, which are relatively unstable, have been compared with respect to their longevity and behavior during aging *in vitro*. These characteristics vary with the isolate used, the temperature of storage of inoculum, the age and kind of source of inoculum, pre- and post-inoculation conditions during the growth of source plants, and dilution. Numerous instances of increasing infectivity during aging indicate the occurrence of a temporary and reversible inhibition of virus. Two isolates, one in each of the green ring mottle and tatter leaf groups, differed from the rest so markedly that they could be classified as strains of an unrelated virus.

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STUDIES ON STONE-FRUIT VIRUSES IN CUCURBIT HOSTS. III. THE EFFECT OF CUCURBIT EXTRACTS ON INFECTIVITY [1]

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It is well-known that naturally-occurring inhibitors in plants cause difficulty in transmitting certain viruses. In the second paper of this series[2], certain anomalies in the behaviour of some stone-fruit virus isolates, during aging *in vitro* experiments, suggested the presence of inhibitors in their cucurbit hosts. The demonstration of the presence of such inhibitors, and their effects on the infectivity of some of the virus isolates is the subject of this paper.

Materials and methods.—The virus material used in this study included all the isolates listed and described in earlier papers [2,3]. For brevity, representative data were drawn only from those obtained with 3 isolates from trees diseased with necrotic ringspot (N.1, N.2, and N.3), 1 isolate from a cherry-yellows tree (Y.2), and 2 isolates from green-ring mottle trees (G.2 and G.3).

In the early tests for inhibition, leaves or portions of leaves of approximately equal area were chosen from diseased plants, and from healthy plants of cucumber (C), vegetable marrow (V), pumpkin (P), and Hubbard squash (S). The healthy tissue will be termed the diluent. The diseased leaf material was ground together with each of the diluents in 2 ml. distilled water. In later experiments, a more comparable method was used in which the diseased material and each of the diluents were ground separately. An aliquot of the infective preparation was then added to an aliquot of each diluent. Water controls were prepared in each experiment by diluting infective material with a quantity of water equal to that of the healthy diluents. The water control preparation could thus be used as a basis for comparing effects of the several diluents in each particular trial.

The preparations were tested for infectivity on cucumber plants immediately after grinding, and again after 4 hrs. at 0°C. The method for assaying infectivity is based upon the total severity of symptom expression in a group of assay plants. This infection rating method has been described fully in an earlier paper[3].

Cucumber was used as the source of virus with all the isolates tested. Whenever material was available, and if vegetable marrow and pumpkin were susceptible to the particular isolate, these 2 species were also used as virus sources. As far as possible, an attempt was made to duplicate experiments with material comparable in age of source of virus, of assay plant, and of diluent tissues, since, as already shown,[2] some of

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[2] Willison, R. S. and Weintraub. 1953. Studies on stone-fruit viruses in cucurbit hosts. II. Some factors affecting the aging of inoculum *in vitro*. Phytopathology 43: 324-328.

[3] Willison, R. S. and M. Weintraub. 1953. Studies on stone-fruit viruses in cucurbit hosts. I. A method of evaluating the infectivity of infectious juice. Phytopathology 43: 175-177.

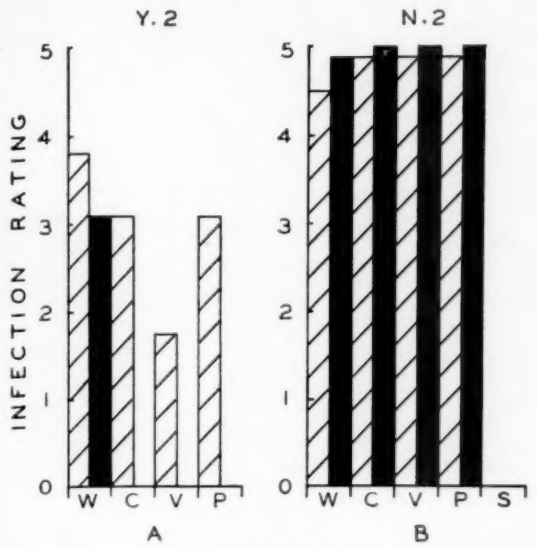

FIG. 1. The effect, on infection rating, of adding aliquots of water (W), and of healthy leaves of cucumber (C), vegetable marrow (V), and pumpkin (P) to : A. isolate Y.2 from cucumber source diseased for 8 days; B. isolate N.2 from cucumber source diseased for 7 days. Crossed bars—inoculation at 0 hrs. Black bars—inoculation after 4 hours at 0°C.

these factors affect the infectivity of the isolates. Experiments were replicated as follows: Isolate N.1, 5 times; N.2, 7 times; N.3, 3 times; Y.2, 4 times; G.2, twice.

It was manifestly impossible, because of limitations of time, space, and materials, to do more than approximate the similarity of conditions in these replications. Consequently, although the magnitude of changes in infectivity was not always reproducible, certain general patterns of behaviour emerged, which are described below.

RESULTS.—*Demonstration of the presence of inhibitors.*—The data with isolate Y.2 (Fig. 1, A) represent the type of result obtained throughout this study, whenever inhibition was encountered. The infectivity of this isolate was inhibited below that of the water control by the addition of the healthy diluents. Significant inhibition occurred both at 0 hours and at 4 hours in the case of the V and P preparations, but was significant only after 4 hours in the C preparation.

Although the addition of the cucumber diluent to isolate Y.2 did not significantly lower the final infection rating of the preparation at 0 hours, it did delay the attainment of that rating. This effect will be discussed more fully below. Thus, when the inhibitors in the curcubit tissues did act against an isolate, the activity was expressed either as a significant drop in the final infection rating of the inoculum or as a delay of full symptom expression and severity on the assay plants.

However, not all isolates were demonstrably inhibited by the addition of the curcubit diluents. Within the limits of the assay method used, isolates N.1 (Fig. 3, A) and N.2 (Fig. 1, B) from young cucumber sources were affected very little by the addition of diluent tissues, with the exception of the Hubbard squash diluent which inhibited completely isolate N.2 both at 0 and at 4 hours (Fig. 1, B). It is of interest that, when healthy cotyledon of Hubbard squash was added instead of the true leaf, inhibition still occurred but to a far lesser degree.

On the other hand, isolate N.3, the source of which was also a necrotic ringspot tree, was found to be much more sensitive to the inhibitors than the other 2 necrotic ringspot isolates. Such a characteristic differentiation marked several of the groups of isolates, certain of the isolates within a group responding to the inhibitors regularly whereas others within the same group did not.

Effect of time on inhibitor activity.—When the inhibitor-virus preparation was allowed to stand for 4 hours at 0°C., 2 types of results were obtained. The first of these is represented by isolate G.2 (Fig. 2, A), which was either not demonstrably inhibited or only slightly so at 0 hours; whereas its infectivity at 4 hours was very markedly depressed. The cucumber and the vegetable marrow diluents had less effect than the pumpkin diluent or the water control (which also, of course, contained cucumber extract and therefore appreciable amounts of inhibitor.) Data obtained from other experiments suggested that certain combinations of inhibitor materials tended to neutralize each other, resulting in a less marked depression of in-

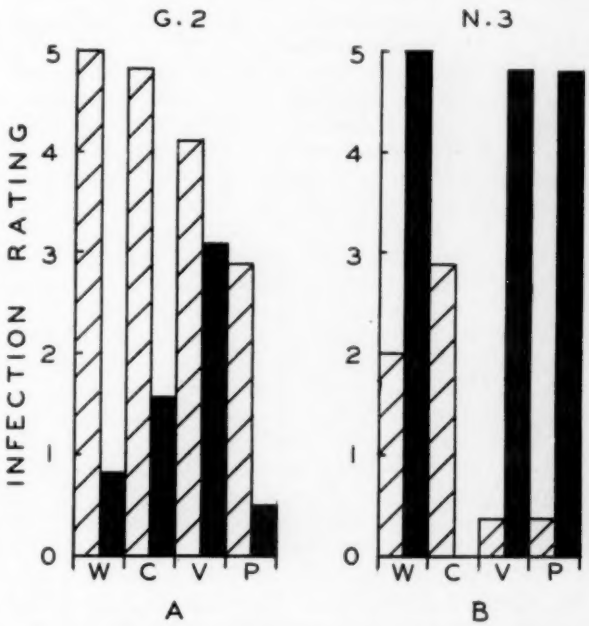

FIG. 2. The effect on infection rating, of adding aliquots of water (W), and of healthy leaves of cucumber (C), vegetable marrow (V), and pumpkin (P) to: A. isolate G.2 from cucumber source diseased for 7 days; B. isolate N.3 from cucumber source diseased for 8 days. Crossed bars—inoculation at 0 hrs. Black bars—inoculation after 4 hrs. at 0°C.

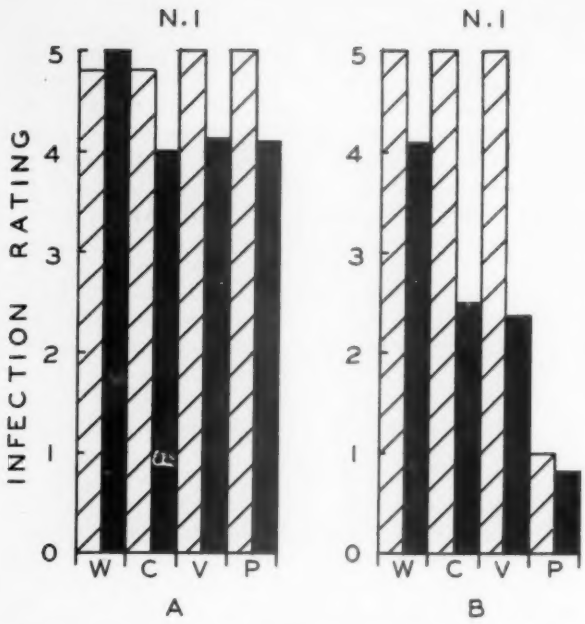

FIG. 3. The effect, on infection rating, of adding aliquots of water (W), and of healthy leaves of cucumber (C), vegetable marrow (V), and pumpkin (P) to: A. isolate N.1 from cucumber source diseased for 11 days; B. isolate N.1 from cucumber source diseased for 25 days. Crossed bars—inoculation at 0 hrs. Black bars—inoculation after 4 hrs. at 0°C.

fectivity.

The G.2 inoculum (water control), however, showed a significant increase in infectivity after 8 hours (Fig. 4 B). A similar effect was also obtained with isolate N.3 in inoculum prepared from infected pumpkin. (Fig. 4 B). This recovery of infectivity after a lapse of time would not be expected if inactivation of the virus proceeded as a first order reaction. Consequently the drop in infectivity at 4 hours is interpreted as being not a part of the normal aging process but as a temporary response to inhibitors present in cucurbit tissues. Subsequently there was a release of active virus after the inhibitors had become inactivated. The sharp rise in infectivity in the preparations of isolate N.3 derived from infected cucumber sources (Fig. 2 B) and the relatively high degree of infectivity of the V preparation of isolate G.2 (Fig. 2 A) can also be explained in the same way, except that in these instances inactivation of the inhibitors occurred during the first four hours of aging.

Effect of age of virus source. —Throughout this study[2], it was evident that, with increased age of source of inoculum beyond a certain point, there was an increased sensitivity to the inhibitors in cucurbit tissues. In an experiment carried out under strictly comparable conditions, the only variable being the age of the virus source, inoculum from the older source (Fig. 3, B) was inhibited considerably more at 4 hours, than the inoculum from the younger source (Fig. 3, A).

Effect of inhibitors on progress of disease.—As already mentioned, the inhibitors may have the effect of delaying symptom expression, although they have no apparent effect on the final, or stabilized, infection rating. The addition of water (W), healthy cucumber leaf (C), and healthy vegetable marrow leaf (V) to inoculum of isolate G.3 made no significant difference in the infection readings on the 14th day, when symptom expression became stabilized. However, readings on the 8th day (Table 1) show that the V assay plants had a significantly lower symptom rating than the W or C assay plants. It is therefore evident that the progress of the disease in this experiment was delayed by the addition of the healthy vegetable marrow tissue.

TABLE 1.—*The effect of 3 diluents on infection rating of isolate G.3, as shown by readings on the 8th and 14th days after inoculation. Diluents: W = water, C = healthy cucumber leaf, V = healthy vegetable marrow leaf*

Diluent	8th day Mean	P	14th day Mean	P
W	3.5 ± .21	<.001	5.0 ± 0.0	<.001
C	3.17 ± .25	<.001	4.83 ± .11	<.001
V	1.0 ± .16	.01±	4.17 ± .52	<.01
Differences				
W–C	.33 ± .33	.5	.16 ± .11	.35
W–V	2.50 ± .28	<.001	.83 ± .52	.30
C–V	2.17 ± .30	.001	.67 ± .53	.40

Similarly, in the example Y.2 mentioned in the first section, a reading on the eighth day after inoculation gave a rating for W = 2.8 and for C = 0.8. On the eighteenth day, the ratings had become more equalized at 3.8 for W and 3.2 for C.

Thermal inactivation of an inhibitor.—In a study of the thermal inactivation of stone-fruit virus isolates, details of which will be reported elsewhere, it was found that the thermal inactivation range of isolate Y.2 was 55°–60°C., for a 10 minute exposure. It was further observed that inoculum exposed at 45°C. produced a higher infection rating than did inoculum exposed at 35°C. or at 40°C. (Fig. 4, A). Since the exposures at the higher temperatures were made before those at the lower ones, this higher infectivity could not be attributed to the previously observed aging of the inhibitor that had resulted in an increase of infectivity with time. It therefore appeared that the inhibitor had itself been inactivated by a 10 minute exposure at 40°–45°C., thereby permitting a higher infection rating for these 2 preparations than for the unheated check or for the preparation exposed at 35°C.

This phenomenon has been observed so frequently and so regularly with certain groups of isolates that its occurrence cannot be considered accidental. With these particular groups of isolates, an increase in infectivity frequently occurs, although not always at the same temperature.

In this particular experiment (isolate Y.2, Fig. 4, A), the leaves used were a dark green color, and it is believed that the inhibitor inactivated at 40°–45°C.

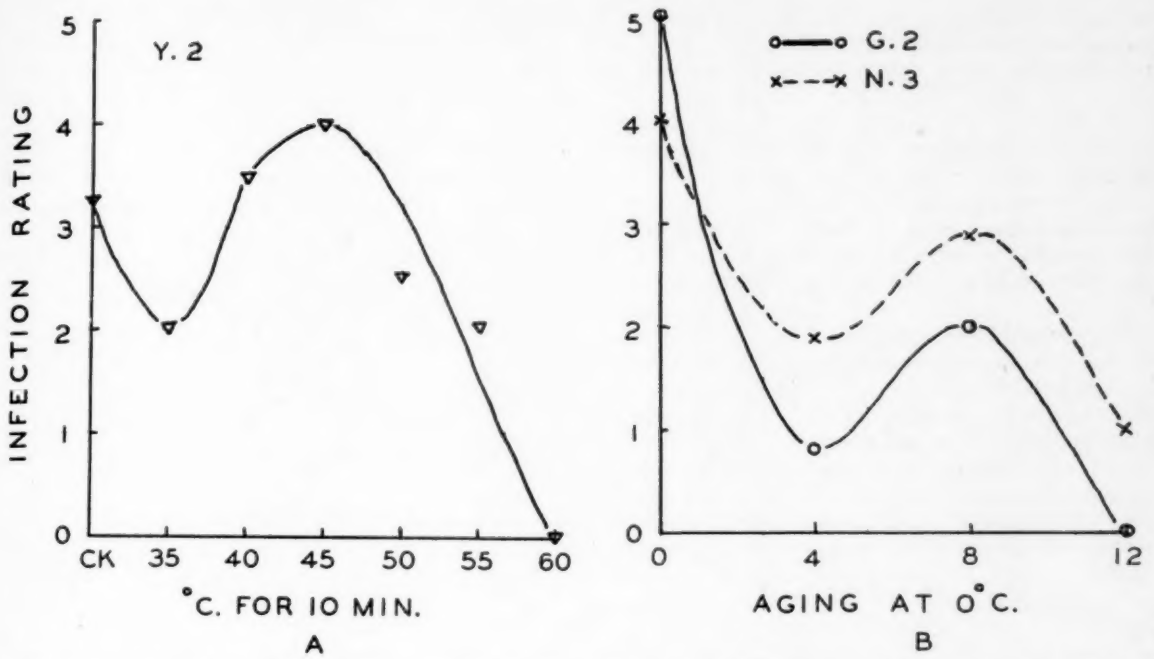

FIG. 4. A) Effect of 10 min. exposure at various temperatures on the infection rating of isolate Y.2. B) Effect of aging *in vitro* at 0°C., on infection rating of isolate G.2 from cucumber source disease for 7 days, and of isolate N.3 from pumpkin source diseased for 23 days.

was associated with the dark green pigment component, or with its formation. This conclusion is drawn from the observation that the Y isolates characteristically gave a low infection rating whenever such dark green leaves were used in the inoculum. Although this dark green pigment appeared less frequently with other isolates, its presence there also resulted in a low infectivity of the inoculum.

DISCUSSION.—No herbaceous hosts, except those in the Cucurbitaceae, have been reported for the stone-fruit viruses. It is therefore evident that studies on the effect of inhibitors from cucurbit tissues must be complicated intrinsically by the constant presence of one or another of the inhibitors. Consequently, it is necessary to proceed on the assumption that, when more inhibitor is added to the virus–inherent–inhibitor preparation, a further depression of infectivity will be observed. That this assumption is valid is borne out by the observation that a depression did occur, although more with some isolates than with others and sometimes complicated by counter-active effects of certain inhibitor combinations.

Other factors make it difficult to determine the exact sensitivity of any one isolate to each of the inhibitors. Age of source of inoculum has already been shown[2] to affect the rate of aging of inoculum. From this it can be deduced that, within limits, the older the source of inoculum, the lower is the infectivity level, and, by inference, the lower the effective virus concentration. Consequently, the effects of the added inhibitors may be more evident with the older sources of inoculum than with the younger (Fig. 3).

Inseparable from this deduction is another that the inhibitor concentration apparently increases with the age of the cucurbit tissue. An instance of this has been noted with isolate N.2, which was totally inhibited by the edition of a young healthy leaf from an old Hubbard squash plant. However, when healthy cotyledon of Hubbard squash was added instead of the true leaf, the inhibitory effect was considerably less. This increase of inherent inhibitor concentration with the age of cucurbit plants may explain, at least partially, the apparent decrease in effective virus concentration in the inoculum from the older source.

Certain behaviour patterns characteristic of specific virus-host complexes in their response to the inhibitors have emerged in this study. It must be realized that these patterns cannot be considered characteristic of any particular isolate or even of any group of isolates. In considering the reaction of an isolate to an inhibitor, it is necessary to take into account not only the stone-fruit source of the isolate and its group, but also the age and species of the cucurbit source of inoculum and the age and species of the source of inhibitor material. Within these limits, however, there have been observed 4 types of behaviour patterns: 1) those in which viruses are sensitive to the inhibitors, as evidenced either by a depression in final infection rating or by a delay in full symptom severity; 2) those in which viruses do not respond demonstrably to the inhibitors, either immediately or 4 hrs. after preparation of the inocula; 3) those in which viruses are only slightly inhibited immediately after preparation, but are considerably more inhibited 4 hours after preparation; 4) those in which

viruses are inhibited to some degree immediately after preparation, but recover their infectivity to a marked and significant extent 4 or more hours after preparation.

The fourth pattern suggests that the longevity of the inhibitors *in vitro* is of short duration. The rise in infectivity after some hours is most plausibly explained by the hypothesis that, after inactivation of the short-lived inhibitors *in vitro*, the virus is released to produce a higher infection rating. This rise in rating is then followed by a falling-off of infectivity as the virus itself ages.

The occurrence of more than 1 inhibitor in any one of the cucurbit species, acting selectively, may also be considered to be a factor. Sill[4] described a cucumber inhibitor of cucumber virus 1 as being effective after 20 days *in vitro*, and stated that, of the cucurbits tested, only watermelon contained a comparable inhibitor. This is at variance with the data presented here since the reversible effect on infectivity, indicating a lessening of inhibitor activity, occurred in a matter of hours rather than days. Furthermore, inhibitors active to a greater or lesser degree against the several stone-fruit viruses appeared to be present in all 4 cucurbits tested. It is therefore possible that different substances are involved in the inhibition of the different isolates. Kuntz and Walker[5] have shown that an inhibitor in spinach is composed of 2 different components, one being active against tobacco mosaic virus, the other against cabbage mosaic virus, and each possessing different physical properties.

The nature of the inhibitors *in vivo* is unknown. However, as already noted, there often appeared to be a correlation between difficult transmission of some isolates, especially those in the cherry-yellows group, and a dark green color of the diseased leaves. This suggests that at least one inhibitory substance is, in some way, connected with a pigment component.

SUMMARY

Inhibitory substances that are capable of producing an initial depression or delay of infectivity of certain stone-fruit virus isolates have been found in 4 cucurbits. This depression may be followed by further reduction of infectivity or by a marked increase in infectivity. Other isolates appear to be insensitive to these inhibitors. The magnitude of the initial depression and the subsequent behaviour of infectivity vary, not only with the isolate itself, but also with the virus-host complex used as the source of inoculum. More than 1 inhibitor may be present, and these inhibitors appear to lose their effect in a few hours, releasing virus in an active form.

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[4] Sill, Webster, H., Jr. 1951. Some characteristics of a virus inhibitor in cucumber. Phytopathology 41: 32.

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A PEPPER RINGSPOT CAUSED BY STRAINS OF CUCUMBER MOSAIC VIRUS FROM PEPPER AND ALFALFA [1]

S. P. Doolittle and W. J. Zaumeyer

Sweet pepper (*Capsicum frutescens* L.) in New Jersey, Maryland, Delaware and adjacent states is affected with a mosaic disease characterized by a mild green mottle of the foliage accompanied by large chlorotic rings and oak-leaf markings on the older leaves and concentric yellow rings on the fruits. These rings may later become necrotic and the fruit spotting occasionally causes appreciable damage in some fields.

A ringspot disease of this type was observed in 1941 by C. M. Haenseler of the New Jersey Agricultural Experiment Station. In 1942 he wrote to the senior author stating that inoculations from pepper to *N. glutinosa* L. had produced symptoms suggestive of those of cucumber mosaic virus (*Marmor cucumeris* Holmes). This isolate, however, had not produced symptoms of ringspot in peppers. Haenseler suggested that the senior author might wish to make a further study of the disease and sent infected pepper fruits. What appeared to be a strain on cucumber mosaic virus was isolated from these specimens but failed to produce ringspot in inoculated peppers. The same was true of inoculations made with juices of the naturally infected peppers.

In 1944, a similar ringspot was collected in Maryland and New Jersey. Inoculations from leaves and fruits resulted in the isolation of tobacco mosaic virus (*M. tabaci* Holmes) and tobacco etch virus (*M. erodens* Holmes) from all the specimens tested. A strain of cucumber mosaic virus also was isolated from some of the same plants. Inoculations were made to peppers with each of the 3 viruses, singly and in all possible combinations but no ringspot occurred (2).

In 1949, a pepper ringspot again was collected in Delaware. In this instance, a single virus was isolated from all fruit or leaf specimens tested. The symptoms were different from any observed in the earlier trials and, on Turkish tobacco, were somewhat similar to those reported by Berkeley (1) in his description of a ringspot of pepper in Ontario caused by a strain of alfalfa virus (*M. medicaginis* var. *capsici* Berkeley). It was found, however, that the virus isolated from the Delaware specimens was a strain of cucumber mosaic virus. While studies of this virus were in progress, the junior author found that mottled leaves of alfalfa, collected in Idaho in 1950, were infected with a complex of a new strain of alfalfa mosaic virus (6) and a strain of cucumber mosaic virus very similar to that isolated from peppers. The latter was separated from the complex by inoculation to cucumbers which are not susceptible to this strain of alfalfa mosaic virus. Comparative studies of the 2 cucumber mosaic viruses have shown that they cause ringspot in peppers and also systemic symptoms in a number of leguminous hosts.

SYMPTOMS AND HOST RANGE.—In these studies, all the inoculations, except for a few inoculations of peppers in the field, were made in the greenhouse at temperatures ranging from 21° to 28° C.

Pepper.—Plants of the California Wonder and World Beater varieties were inoculated. Both show similar symptoms but there is less fruit spotting on World Beater. The 2 strains cause symptoms of the same type on both varieties. The original virus complex from alfalfa also produces symptoms on pepper much like those caused by its cucumber mosaic virus component. The alfalfa virus component alone, however, produces no symptoms on pepper.

Pepper plants inoculated with either the pepper or the alfalfa strain of cucumber mosaic virus first show circular, yellow-green spots on inoculated leaves. These spots have a slightly darker margin and a center with minute concentric markings. They are about 2 mm. in diameter and there is a yellowing of most of the leaf. On young plants, infected leaves soon drop but on large plants they persist for some time. Soon after inoculation systemically invaded leaves develop pale green, concentrically ringed spots 2–3 mm. in diameter. These coalesce and produce an intricate mottled pattern. Within 2–3 weeks after infection, some of the older leaves show circular spots about 6–8 mm. in diameter with pale green concentric markings. Such spots soon are surrounded by a broad band of yellow-green with a diffuse margin (Fig. 1F). As the yellow band develops, the concentric markings in the center become very faint and the spots appear as wide, yellow-green rings. Usually, only 1–3 spots occur on a leaf. Some leaves are not ring-marked but develop pale green oak-leaf markings. Both the ring and the oak-leaf patterns eventually become necrotic and the leaf drops. These symptoms have not been observed on small plants. In late stages of the disease, the leaves are only faintly mottled with flecks of light green, are slightly misshapen, and are more pointed than those of healthy plants. There is little leaf malformation in early stages of the disease. The alfalfa strain produces a milder mottling and less ringspotting of the older leaves than the strain from pepper.

Young fruits of infected plants generally show no evidence of disease although occasional ones have pale green blotches of irregular shape. As the fruits approach the mature green stage, some are marked with concentric ringspots about 8–20 mm. in diameter (Fig. 1E). These are similar to those on the leaves but there is no later development of a yellow band around the concentric markings. The spots apparently do not develop at the points where pale green blotches

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occurred when the fruit was small. The ringspots may remain yellow and the centers become slightly depressed or the rings may become brown and depressed at the margins. Both the pepper and the alfalfa strains produce markings of the same type on the fruits. Similar symptoms occur on plants inoculated either in the greenhouse or field.

Tobacco, Nicotiana tabacum L. var. Samsun.—On Turkish tobacco both strains cause grayish-white, circular local lesions 3–5 mm. in diameter (Fig. 1 H,I). These may be broad rings with small centers or solid spots. Some leaves show grayish-white oak-leaf markings. Leaves just above those inoculated develop a pronounced yellow green veinbanding but the later growth shows only a green stippled mottle that becomes mild as the plants grow older but is never masked. Etch or necrosis occurs only on inoculated leaves. The alfalfa strain produces smaller spots and less oak-leaf pattern than the pepper strain.

N. glutinosa. Both strains occasionally produce tan, circular, necrotic lesions on the older systemically infected leaves. These are more common with the pepper strain. Both strains produce systemic green veinbanding and mottling with small spots of light green.

N. rustica L. Both strains produce white etched rings on inoculated leaves. Systemic mottling is mild but some leaves show large dark green blistered areas.

Datura stramonium L. Circular, zonate, yellow-green local lesions with a definite margin are produced by both strains (Fig. 1 J). No systemic infection occurs.

Tomato, *Lycopersicon esculentum* Mill. var. Marglobe.—Inoculated leaves become yellowed and show a brown, necrotic stippling. Systemic mottling is mild and there is no necrosis. The plants are stunted but the leaves are not markedly deformed. Symptoms caused by the pepper strain are more pronounced than those caused by the alfalfa strain.

Eggplant, *Solanum melongena* L. var. Black Beauty.—Both strains produce large yellow local lesions. Systemic infection does not occur.

Cucumber, *Cucumis sativus* L. var. A and C, Tokyo Long Green and Yorkstate Pickling.—In A and C plants, inoculated with either strain, the first systemically infected leaves show yellow dots that enlarge and form asteroid spots. Later growth sometimes shows asteroid spotting but more often is mottled in a pattern like that caused by the type strain of cucumber mosaic virus (Fig. 1 G). Plants of a strain of Tokyo Long Green that is highly resistant to type cucumber mosaic virus show no symptoms when inoculated with the pepper or the alfalfa strain. Plants of Yorkstate Pickling, which is tolerant of cucumber mosaic virus, show a mild mottling in leaves developing at the time of inoculation but little evidence of disease in later growth. The symptoms are the same with both strains and with the type strain of cucumber mosaic virus.

Spinach, *Spinacia oleracea* L. vars. Longstanding Bloomsdale and Old Dominion.—Both strains produce systemic mottling, dwarfing, and killing of the leaves of Longstanding Bloomsdale, which is susceptible to the typical cucumber mosaic virus. The alfalfa strain only causes systemic mottling and occasional death of plants of the variety Old Dominion, which is resistant to the typical cucumber mosaic virus.

Bean, *Phaseolus vulgaris* L.—Twelve varieties of bean were inoculated. Both strains produce systemic mottling of a similar type on all these varieties but the mottling varies in intensity with variety. On 8 of the 12 varieties, the mottling caused by the alfalfa strain is much more pronounced than that produced by the pepper strain. Necrotic lesions are not produced on any variety by either strain.

The first symptoms consist of marked epinasty of the first unifoliate leaves. On Idaho Pinto, Landreth Stringless Green Pod, and Michelite there is a yellow veinclearing that does not appear in other varieties tested. Systemic mottling of a pronounced type occurs on the varieties just mentioned (Fig. 1B) and on Bountiful and Ideal Market. On Great Northern U.I. Nos. 16 and 123, Black Valentine, Red Mexican, U.S. Refugee No. 5, and Tendergreen the mottling caused by the alfalfa strain varies from medium to mild. On plants infected with the pepper strain, the mottling is less marked in all varieties except Black Valentine, Red Mexican, and the 2 strains of Great Northern.

Tepary bean, *P. acutifolius* var. *latifolius* Freeman.—Both strains produce small, reddish brown local lesions and occasional veinal necrosis on inoculated leaves but no systemic infection (Fig. 1 A). More lesions are produced by the alfalfa strain.

Lima bean, *P. lunatus* L. var. Henderson Bush.—Leaves inoculated with the alfalfa strain develop many circular, grayish-white, necrotic local lesions and some veinal necrosis. There is a strong systemic mottle followed by petiole and stem necrosis that sometimes kill the plants. The pepper strain produces a few faint lesions on inoculated leaves and a mild systemic mottle but no petiole or stem necrosis.

Rice bean, *P. calcaratus* Roxb. A systemic mottle is caused by both strains.

Broad bean, *Vicia faba* L. var. Broad Windsor.—Both strains produce brown local lesions 1–2 mm. in diameter. The alfalfa strain produces systemic spotting of the leaves with brown, necrotic spots and in some plants it causes stem necrosis. The pepper strain produces necrotic ring markings and little stem necrosis (Fig. 1 C).

Yardlong cowpea, *Vigna sesquipedalis* (L.) Fruwirth.—The alfalfa strain causes yellow rings on inoculated leaves and a distinct systemic mottle accompanied by malformation of the leaflets (Fig. 1 D). The pepper strain causes similar but much milder symptoms.

Cowpea, *Vigna sinensis* (Torner) Savi vars. Blackeye and Victor.—The symptoms produced by the alfalfa strain are similar to those it causes on yardlong cowpea but there is little malformation of the leaflets. The pepper strain causes faint mottling and no distortion of the leaflets.

Pea, *Pisum sativum* L. vars. Laxton Progress, Early Perfection and Perfected Wales.—Both strains cause

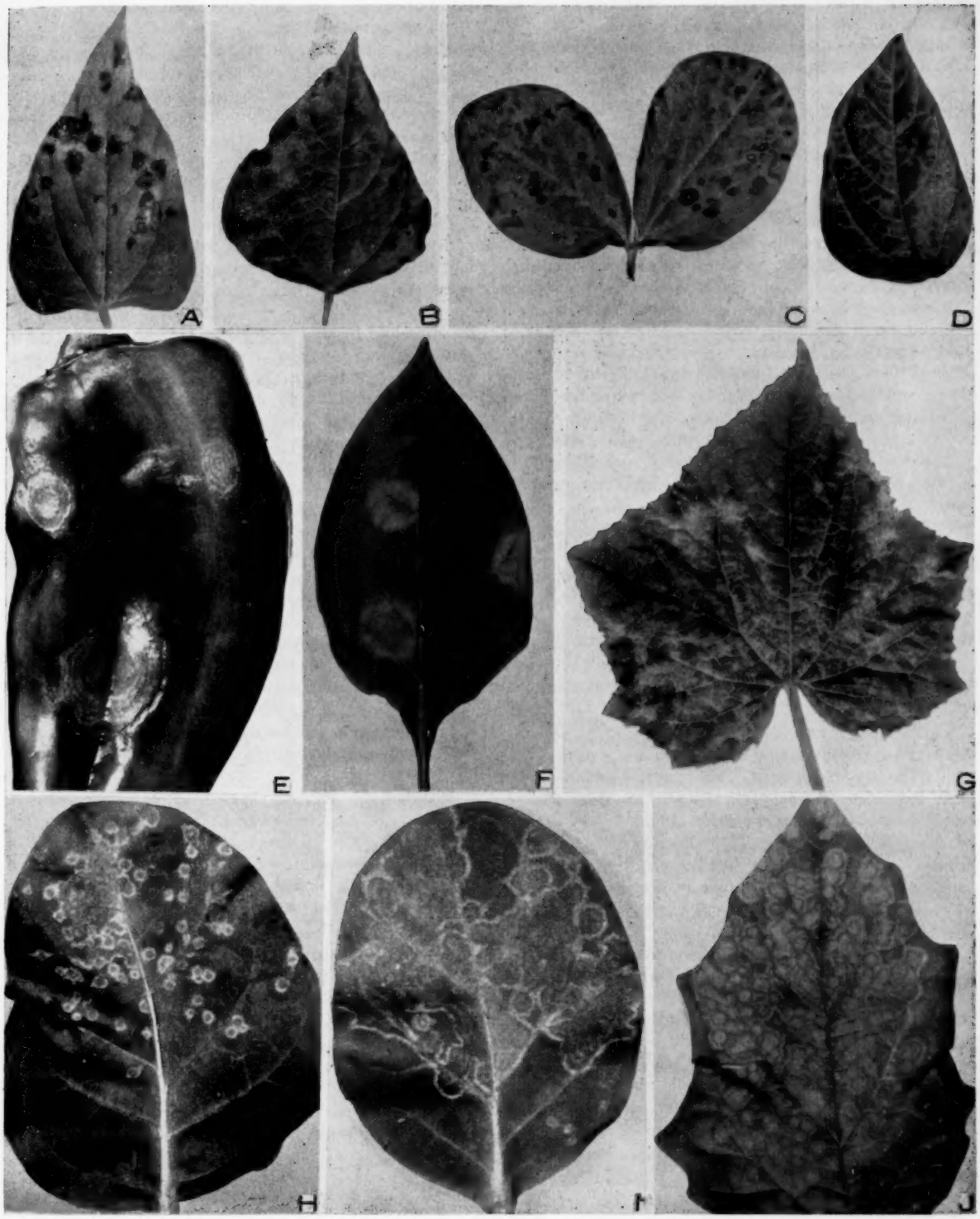

FIG. 1. Symptoms induced in various host plants by strains of the cucumber mosaic virus causing ringspot in pepper: by the alfalfa strain, A and H, local lesions in tepary bean and Turkish tobacco respectively; B, D, and G) systemic mottle in Idaho Pinto bean, Blackeye cowpea, and A and C cucumber, respectively; by the pepper strain, E and F, ringspotting of fruit and leaf of World Beater pepper; C, I, and J) local lesions in broad bean, Turkish tobacco, and *Datura stramonium*, respectively.

withering of the inoculated leaves, mild systemic mottle, and occasional stem necrosis.

Sweet pea, *Lathyrus odoratus* L. var. Mars.—Both strains cause symptoms similar to those on pea but stem necrosis is more common and there is little systemic mottling of the leaflets.

White sweet clover, *Melilotus alba* Desr.—Both strains cause an intense yellow-green systemic mottling and occasional small, green, ring markings.

Crimson clover, *Trifolium incarnatum* L.—Both strains cause pronounced dark green, striped mottle on systemically infected leaves.

Other species. Both strains produce symptoms similar to those caused by the typical cucumber mosaic virus on *Petunia hybrida* Vilm.; celery, *Apium graveolens* L. var. Golden Self Blanching; sweet corn, *Zea mays* L. var. Golden Bantam; snapdragon, *Antirrhinum majus* L. var. Tall Giant; zinnia, *Zinnia elegans* Jacq. var. Golden Queen; and globe amaranth, *Gomphrena globosa* L.

No infection was obtained with either strain on cabbage, *Brassica oleracea* L. var. Jersey Wakefield; alfalfa, *Medicago sativa* L.; red clover, *Trifolium pratense* L.; white clover, *Trifolium repens* L.; China aster, *Callistephus chinensis* (L.) Necs var. Crego; or garden balsam, *Impatiens balsamina* L.

Properties.—Both strains remained active after heating for 10 minutes at 70° C. but were inactivated at 75° C. Infection was obtained with both strains at dilutions as high as 1 to 10,000 and both withstood aging *in vitro* for 4 days at 18° C. These properties correspond closely with those of the typical strain of cucumber mosaic virus.

Cross protection.—When zinnia plants showing marked systemic symptoms after inoculation with either the pepper or the alfalfa strain were inoculated with Price's test strain (No. 6) of cucumber mosaic virus, no lesions were produced on the leaves of any of 25 plants infected with either strain. Necrotic lesions occurred on all the healthy check plants inoculated with the test virus. This protection effect indicates that both the pepper and the alfalfa virus are strains of cucumber mosaic virus.

Leaves of Idaho Pinto and Bountiful beans, which showed systemic mottling with either the pepper or the alfalfa strain of the cucumber mosaic virus, were inoculated with a typical strain of alfalfa mosaic virus which produces necrotic lesions but no systemic symptoms on beans. All the inoculated leaves showed typical lesions of the alfalfa mosaic virus. This indicates that the virus strains studied are not of the alfalfa mosaic group.

Discussion.—The ringspotting produced on peppers by the strain of the alfalfa mosaic virus described by Berkeley appears to be somewhat like that produced by the strains of cucumber mosaic virus isolated from pepper and alfalfa. These strains, however, differ from *M. medicaginis* var. *capsici* in their effect on a number of hosts and in their thermal inactivation point.

Berkeley's alfalfa mosaic virus strain produces necrotic local lesions on bean but no systemic symptoms, whereas the 2 strains of cucumber mosaic virus herein described produce no local lesions and are systemic on all bean varieties tested. The alfalfa mosaic virus also differs in its production of systemic symptoms on *Datura stramonium* and eggplant, and in its failure to cause systemic infection of tomato. On snapdragon, the strains here described do not cause the reddish rings or patterns described by Berkeley. On tobacco, both alfalfa mosaic virus and the strains of cucumber mosaic virus from pepper and alfalfa produce grayish-white necrotic rings on inoculated leaves but cucumber mosaic virus does not cause the systemic necrotic rings and flecks described by Berkeley. Alfalfa mosaic virus is inactivated at 64° C. while those described herein are infective after heating at 70° C. It appears, therefore, that the virus causing a ringspot of pepper in Ontario is not similar to the strains we have found to cause a similar disease in Maryland, Delaware, and New Jersey.

On non-leguminous hosts, the symptoms produced by the strains herein described are, on the whole, quite similar to those produced by the typical strain of cucumber mosaic virus. The physical properties also conform with those of the type strain. These characteristics, plus the evidence of cross-protection against Price's test strain on zinnia, and the fact that the Tokyo Long Green cucumber, which is highly resistant to the type strain, is also resistant to the pepper and alfalfa strains, identified the pepper and alfalfa isolates as related strains of cucumber mosaic virus. However, the systemic infection of the resistant Old Dominion spinach by the alfalfa strain and the lack of such infection by the strain from pepper constitute differences that prevent classing the isolates as a single strain.

A difference also has been evident in the relative intensity of symptoms produced by the 2 strains on leguminous and solanaceous hosts. On susceptible legumes both strains produce symptoms of a similar type but those produced by the alfalfa strain have been distinctly more pronounced on almost all such hosts on which systemic infection occurred. On a number of solanaceous hosts, however, the pepper strain produces more pronounced symptoms than those produced by the alfalfa strain although these differences are less marked than those on leguminous hosts.

The first instance of systemic infection of bean by a strain of cucumber mosaic virus was that described by Whipple and Walker (5), who recovered such a strain from pea and bean and designated it as No. 14. This strain differs from the strains herein described in several respects. It did not produce grayish-white, circular, local lesions on tobacco, and the systemic symptoms on this host were quite different from those produced by the strains described herein. It also differed in its production of systemic symptoms on *Datura stramonium* and the lack of systemic infection on broad bean. Fulton (4) isolated 5 strains of cucumber mosaic virus from spinach; 4 of these produced systemic infection in bean. These strains differ

from those described herein in their failure to produce local lesions on tobacco and in the type of systemic symptoms they produce on this host. On tomato, they apparently cause more leaf distortion than the strains herein described.

Frequently ringspotting does not occur on fruits of pepper plants inoculated with the strains of cucumber mosaic virus herein described. This lack of fruit symptoms does not appear to be related to the age of the plant or of the fruits at the time of inoculation and it is possible that environmental factors may affect this expression of the disease. If such a factor is involved, it may be that earlier failures to produce ringspot with strains of cucumber mosaic virus isolated from diseased pepper were due to the effect of environment on the susceptibility of the fruits. If this is the case, it is possible that other strains than those herein described may produce similar ring markings on the fruits.

In isolations made from pepper in 1944, tobacco etch and tobacco mosaic viruses were recovered from each of 8 specimens of ringspotted leaves or fruits tested. A cucumber mosaic virus was recovered from only 2 of these specimens and at that time it was thought that the etch virus might be concerned in the production of ringspot symptoms. This hypothesis could not be substantiated and it is possible that a cucumber mosaic virus was present in all the plants and that the presence of the other viruses led to an interference effect which reduced the potency of the cucumber mosaic virus and made it difficult to isolate. Such a theory receives some support from the fact that, in peppers artificially inoculated with these 3 viruses, it sometimes is difficult to recover the cucumber mosaic virus component from the complex.

SUMMARY

Pepper in Maryland, Delaware, and New Jersey and adjacent States is affected with a virus disease characterized by ring markings on the leaves and fruits. This disease is caused by a strain of cucumber mosaic virus. It also may be caused by a very closely related strain recovered from alfalfa leaves collected in Idaho, where it occurred in combination with a new strain of alfalfa mosaic virus.

Both strains of cucumber mosaic virus produce similar characteristic lesions on Turkish tobacco and both cause systemic infection on bean, white sweet clover, crimson clover, cowpea, pea, and sweet pea. Both produce similar symptoms on all the hosts tested except Old Dominion spinach, on which infection has occurred with the alfalfa strain but not with the strain from pepper. The alfalfa strain causes more pronounced symptoms on nearly all the leguminous hosts tested, while the pepper strain causes more severe symptoms on a number of solanaceous hosts.

The physical properties of the 2 strains agree with those of the type strain of cucumber mosaic virus. On zinnia, both strains protect against Price's test strain of cucumber mosaic virus.

These strains differ in host range and symptoms from other strains of cucumber mosaic virus known to cause systemic symptoms on beans.

The strain of alfalfa mosaic virus that causes a ringspot of pepper in Ontario differs from the ringspot strains of the cucumber mosaic virus in its host range, symptoms produced on some hosts, and thermal inactivation point.

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RESISTANCE OF POTATO TUBERS TO CERTAIN PARASITIC FUNGI [1]

J. N. Mishra [2]

Reviewing the mechanism of resistance in plants, especially to parasites of the facultative type, Brown (4) postulated 2 ways in which a parasite may utilize the food reserve contained within the double barrier of a mechanically strong cell wall and a relatively impermeable plasma membrane: A) Mechanical penetration which may kill the living cells and result in a serious disturbance in the metabolism of the plant; B) Chemical action by which a pathogen brings about killing in advance and then lives saprophytically on the killed tissue. The chemical process of attack of a plant tissue by a pathogen seems to be the most general. It applies to bacterial plant pathogens and to fungi, such as *Botrytis cinerea* Pers., which secrete vigorously acting pectic enzymes. The capacity of a fungus to produce pectic enzymes under nutrient conditions approximating those at the wound surface of plant tissue, does not necessarily mean that the fungus is able to parasitize such tissues under normal conditions. Thus *B. cinerea*, which is not normally a parasite of potato tubers, produces active pectic enzymes in killed potato tissue, whereas *Pythium debaryanum* Hesse, which is a normal parasite of potato does not do so. Hawkins and Harvey (10) and Abdel-Salam (1) have suggested that parasitization of potato and lettuce tissues by *P. debaryanum* is through mechanical penetration of the cell walls. In the present investigation the probable physical and physiological conditions under which *B. cinerea* can be made to attack potato tissue have been examined with reference to those conditioning the attack of the tissue to *P. debaryanum*.

Materials and methods.—For inoculation experiments mostly tubers of the Majestic variety of potato were used. Spores of *B. cinerea* from 10–15-day old cultures on potato-dextrose agar were introduced in 1.5 cm. deep cavities. Four-6-day old mycelial inocula of *P. debaryanum* on the same medium were introduced in similar cavities dug out with a cork-borer (No. 2 size). In all cases cavities were sealed with vaseline after inoculation. The amount of attack was determined by weighing the rotted tissue after 7 days' incubation at 20°C.

Determination of enzymic activity.—Brown's (5) method of noting the time taken for the coherence loss of potato tuber discs (15 mm. in diameter and 0.5 mm. thick) immersed in the culture filtrate containing the enzyme, was used for the purpose.

Preparation of the pectic enzyme.—To obtain an active enzyme preparation from *B. cinerea*, 10 cc. of the synthetic medium (glucose 25.0 gm., asparagine 10.0 gm., potassium dihydrogen phosphate 5.0 gm., magnesium sulphate hydrated 2.5 gm. and water to 1 liter) as used by Ashour (3), were seeded with spores and the culture fluid containing the enzyme after 3 days' growth at 20°C was used. An active enzyme preparation from *P. debaryanum* was obtained by growing the fungus for 10 days at 20°C in a medium prepared on the basis of the composition of potato tuber tissue (glucose 1.0 gm., peptone 0.9 gm., asparagine 0.9 gm., starch 19.5 gm., potassium chloride 0.41 gm., tribasic potassium phosphate 0.517 gm., magnesium sulphate hydrated 0.197 gm., magnesium chloride hydrated 0.075 gm., sodium chloride 0.049 gm., calcium chloride anhydrous 0.052 gm., ferric chloride 0.024 gm., and water to 75.8 cc.) as given in Thorpe's Dictionary of Chemistry. The concentrated medium to which only 1.0 gm. of starch was added, had to be diluted 12 times prior to inoculation in order to obtain a very active (maceration time 15 minutes) enzyme preparation.

Experiments and results.—*Effect of maturity of tubers.*—To test the effect of maturity on susceptibility, tubers were inoculated periodically from early June when they were just big enough to be handled. Great Scot and Epicure tubers were also used but rather late in the season when almost mature. In some experiments a set of Majestic tubers was inoculated soon after lifting and another set from the same stock, after 21 days' storage at room temperature, to observe whether the effect of prematurity would persist. The results are in Table 1.

Table 1.—*Effect of maturity on susceptibility of potato to P. debaryanum and B. cinerea*

Variety	Approx. diameter cm	Rot in gm. Pythium	Botrytis
		In fresh tubers	
Majestic	3.8 × 2.5	0.346 ± 0.094	0.521 ± 0.114
do	6.3 × 5.0	0.390 ± 0.062	0.540 ± 0.141
do	8.8 × 6.3	0.121 ± 0.042	0.073 ± 0.018
Epicure	8.6 × 6.3	0.126 ± 0.032	0.070 ± 0.021
Great Scot	6.3 × 6.3	0.085 ± 0.022	0.045 ± 0.019
		In stored tubers	
Majestic	6.3 × 5.0	0.138 ± 0.016	0.048 ± 0.012

Immature tubers inoculated soon after lifting are susceptible to *B. cinerea*, but the susceptibility vanishes after storage for a period at room temperature. *P. debaryanum* behaves similarly but its attack persists at a much lower level. Epicure and Great Scot appear to be much less susceptible than Majestic.

Effect of sprouting.—Gregg (9), observed increased susceptibility of sprouting tubers to *Bacterium aroideae*. To induce sprouting, the late sprouting Majestic tubers were treated in the following ways as

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[2] The author is grateful to Professor W. Brown, F.R.S. for suggesting the problem and for his help. This work was done through a scholarship from the Government of Bihar for which gratitude is expressed.

recommended by Appleman (2) and Denny (7): A) Peeling; B) exposure to ethylene chlorhydrin in a closed space at the rate of 0.02 cc. of 90 per cent strength per liter volume, higher concentrations having an inhibitory effect on sprouting at the time; C) soaking in 0.5 per cent thiourea solution. The treated tubers were stored under moist sawdust at 20°C. Two controls, one under dry sawdust and the other under moist sawdust were similarly stored. All tubers except those put under dry saw dust, which were very much flaccid, remained turgid and sprouted well. To observe the relationship between water content and rot, the water content of each potato was determined after collecting its rot. Rotting due to *B. cinerea* was negligible. The average water content and rot due to *P. debaryanum* in each treatment are in Table 2.

TABLE 2.—*Effect of sprouting on susceptibility of potato to P. debaryanum* [a]

Treatment	Water content per cent	Rot in gm.
Dry control	75.61 ± 1.16	2.417 ± 1.586
Wet control	77.85 ± 0.52	0.238 ± 0.110
Ethl. chlor.	85.37 ± 1.06	0.227 ± 0.107
Sod. thio.	78.76 ± 0.47	0.350 ± 0.261
Thiourea	77.75 ± 0.69	0.291 ± 0.217
Peeling	78.49 ± 1.42	0.246 ± 0.101

[a] The value of 't' for the difference between the control mean and the lowest treatment mean is 1.3, and that required for P = O .05 is 2.1.

From Table 2 it appears that there is no difference between the treatments and the wet control. The dry control appears to give much more extensive rot but the difference between it and the treatments is insignificant because of a large standard error.

To test in more detail the effect of sprouting on susceptibility, quick sprouting of freshly harvested Majestic tubers was obtained by storing them for 7 days in 5 per cent oxygen and 95 per cent nitrogen after the method of Thornton (11). No effect of sprouting was noted, the amounts of rot due to *P. debaryanum* were: treatment 0.186 ± 0.054 and control 0.212 ± 0.042 g. *B. cinerea* was completely inactive.

Effect of prestorage at 35°C.—Vasudeva (12) and Chona (6) noticed increased susceptibility of apples to certain parasitic fungi after storage at high temperature, and Fernando (8) and Gregg (9) noticed a similar effect of bacteria on preheated tubers. In some experiments the susceptibility of 3 varieties of Potato stored at 35°C. for 7 days was examined, but no effect of preheating was observed. *B. cinerea* was inactive. The results are in Table 3.

Effect of water content.—Water content of plant tissue has generally been found to be associated with increased susceptibility to parasites. A high negative correlation (r = −0.3482) between normal water content of potato and rot produced in it by *P. debaryanum* was observed in Table 2. The effect of water content higher or lower than normal was then investigated. To facilitate absorption of water, potatoes were uniformly peeled. They were weighed individually, numbered and put in a series of jars connected to one suction pump. One set was kept in an empty jar as control, another over fused calcium chloride separated by a wire gauge, and a third in sterile distilled water. After pumping for 2 hours the tubers were weighed again. Thus a change of ± 6-to-10 per cent of the water content could be obtained. A set of 10 tubers which had absorbed water was desiccated over fused calcium chloride back to the original weight. Thus 4 sets of tubers were available for inoculation. The results are in Table 4.

TABLE 3.—*Susceptibility of preheated tubers to P. debaryanum*

Variety	Rot in gm. 15°C	35°C
Majestic	0.188 ± 0.061	0.210 ± 0.058
Great Scot	0.096 ± 0.026	0.122 ± 0.046
Epicure	0.138 ± 0.076	0.126 ± 0.054

B. cinerea produced a typical soft rot on tubers whose water content had been raised. No change in tissue during absorption seems to be involved as on desiccation to the original water content, susceptibility is lost completely.

Behaviour of pectic enzymes.—To compare the behaviour of the enzyme preparations of the test fungi with that of the organisms, solutions of exoenzymes of the fungi were prepared as described earlier. Two-tenths of a cc. of the preparations were pipetted into the cavities of standard depth on tubers which had been allowed to desiccate or absorb water as described above. The tubers were incubated for 68 hours at 20°C, after which the rot was measured. The measures of enzymic activity of *P. debaryanum* and *B. cinerea* as determined by Brown's (5) method were 15 and 20 minutes respectively. Results are in Table 5.

In every case a typical soft rot was produced. The performance of both preparations of nearly equal initial activity was identical in every case, the water content directly conditioning attack. Comparison of the controls in Tables 5 and 6 reveals that *P. de-*

TABLE 4.—*Effect of increased water content on susceptibility of potato to P. debaryanum and B. cinerea*

Treatment	Change in water content, per cent	Rot in gm. Pythium	Botrytis
Control	0	0.120 ± 0.042	0
Desiccation	−6.5	0.134 ± 0.062	0
Absorption	8.8	0.118 ± 0.026	3.540 ± 0.471[a]
Absorption & Desiccation	0.6	0.141 ± 0.054	0

[a] The rot by *B. cinerea* was measured after 3 days only.

TABLE 5.—*Rotting of potato tissue by pectic enzymes*

Treatment	Rot in gm.	
	Pythium enzyme	Botrytis enzyme
Control	0.335 ± 0.081	0.312 ± 0.013
Desiccation	0.085 ± 0.016	0.065 ± 0.014
Absorption	5.550 ± 0.582	6.480 ± 0.614

baryanum does not secrete any appreciable quantity of the pectic enzymes on ordinary potato tissue and, therefore, the question of their inactivation does not arise.

DISCUSSION.—*P. debaryanum* generally attacks soft and meristematic tissue and a number of workers (1, 10) have shown that the attack is mechanical. From the present work, mainly directed towards chemical aspect of attack, similar conclusions can be drawn. *P. debaryanum* produces pectic enzymes in synthetic media resembling the composition of potato at a very high dilution in which very feeble growth of the fungus takes place. The culture fluid had to be incubated for at least 8 days before any pectic enzyme could be detected by the maceration test. Hawkins and Harvey (10) found that after wounding the surface of potato, if inoculation is delayed for 3 hours no attack takes place. It was also found that no attack ensues even if the fungus is introduced in a water injected potato, but there is very heavy rotting in an injected potato on introducing a small quantity of the pectic enzyme produced by the fungus.

B. cinerea which is normally a parasite of foliage and soft fruits behaves differently on potato tissue. It readily secretes pectic enzymes in synthetic and vegetable media. But though the fungus seems to be normally provided with the mechanism of enzymic attack, it cannot parasitize normal potato tissue. There is evidence to suggest that the fungus, unlike *P. debaryanum*, produces pectic enzymes in normal potato tissue, but its diffusion is very much restricted. If the fungus is introduced in a water injected tuber there is heavy rotting in a very short time, the pectic enzyme of the fungus behaving exactly in the same way. Extensive rotting also takes place in immature tubers whose water content is naturally higher than in mature tubers.

SUMMARY

The nature of parasitization of potato tissue by *P. debaryanum* and *B. cinerea* was investigated. It is suggested that *P. debaryanum* does not produce pectic enzymes in potato tissue under any condition and its attack is a mechanical one.

B. cinerea which is not a normal parasite of potato was found to produce pectic enzymes in potato tissue, but its attack is restricted because of lack of diffusion of the enzyme into the normal tissue. It is suggested that parasitization of plant tissue by *B. cinerea* involves an enzymic process.

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ISOLATION OF COMPATIBILITY TYPES OF ENDOCONIDIOPHORA FAGACEARUM FROM OAK WILT TREES NATURALLY AND ARTIFICIALLY INOCULATED [1]

H. L. Barnett and John M. Staley [2]

One of the major unsolved phases of the oak wilt problem is the method of infection in nature. Both conidia and ascospores of *Endoconidiophora fagacearum* Bretz are known to be capable of producing the disease following artificial inoculation, but it is not known whether both kinds of spores commonly serve as inoculum in nature. Mycelial mats bearing abundant conidia and frequently producing large numbers of perithecia and ascospores have been found beneath the loosened bark on dead oak wilt trees. It has been suggested that these mats serve as the principal source of inoculum (3, 4, 7).

Unpublished work from this laboratory involving 96 isolates from 10 states has shown that both compatibility types are present in all oak wilt areas. Few naturally infected trees have yielded isolates of both compatibility types, which are designated as A and B by Hepting *et al* (6). There has been no satisfactory explanation for this situation. Hepting *et al* (5) reported that 1 tree at Asheville, North Carolina, gave mixed cultures of A and B mycelia.

If infection occurs with ascospores or a mixture of A and B conidia, it would be assumed (in the absence of evidence to the contrary) that both types of spores have equal opportunity to be spread throughout the tree. A high percentage of naturally infected oak wilt trees yielding isolates of both A and B types might be considered as evidence of infection with ascospores or mixtures of conidia. On the other hand, isolation of only 1 type of mycelium from an adequately sampled tree could indicate either that infection resulted from conidia of 1 type, or that only 1 type survived in the tree. Up to the present there has been little evidence to support either view.

The purpose of this paper is to report results of extensive isolations from naturally and artificially inoculated oak trees and to present evidence that in oak trees inoculated with mixed isolates of *E. fagacearum*, only 1 isolate frequently survives.

MATERIALS AND METHODS.—Isolates of the oak wilt fungus were obtained through routine sampling of suspected oak trees in West Virginia during 1952. Seventy-three isolates obtained in this way from 22 trees were included in this study. To eliminate variation due to differences in method of sampling, 21 additional naturally infected trees were more adequately sampled by the same person. From 99 of these samples, 400 isolates, each from a different chip, were used.

FIG. 1. Diagram of red oak tree No. 2 artificially inoculated with ascospores at 0. Two months later samples were taken from 13 points marked X.

In the second part of this work red oak trees were inoculated with mixtures of conidia of A and B compatibility types and with ascospores obtained from crosses in culture. Inoculations were made by placing a spore suspension in a chisel cut in the trunk about

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[2] The authors gratefully acknowledge the help of Foster Brown, student laboratory assistant.

TABLE 1.—*Compatibility types of isolates from red oak trees artificially inoculated with mixed spores of A and B types. Tree 13 was inoculated May 22; the others on July 15. Samples were taken in September and October*

Tree No.	Tree Size	Inoculum Used	Trunk Samples	Branch Samples	Isolates Used	A Isolates	B Isolates
1	24 in.	Mixed conidia 643A + 633B	0	9	18	0	18
2	Forked 8, 6, 3 in.	Ascospores 845A × 613B	4	9	40	40	0
3	Forked 12, 7 in.	Ascospores 875A × 613B	0	13	26	26	0
4	7 in.	Ascospores 638A × 862B	0	10	20	20	0
6[a]	10 in.	Mixed conidia 643A + 633B	0	2	6	0	6
13	8 in.	Mixed conidia 805A + 633B	2	1	10	9[b]	0

[a] Duplicate of tree No. 1.
[b] One culture was found to be a mixture of A and B.

4 ft. from the ground. After oak wilt symptoms were quite pronounced and before the trees were completely dead, samples were taken from several parts of each tree. The approximate locations of samples taken from tree No. 2 are shown in Fig. 1.

Isolations were made by placing chips of oak wood on glucose-phenylalanine agar (1). A total of 116 isolates representing 48 samples from 6 artificially inoculated trees was used in these experiments.

The compatibility type of each isolate was determined by growing the cultures for 7 days at 25° C. on dilute maltose-Casamino Acids agar (2). The cultures were then spermatized by conidia of known A and B isolates. If the 2 isolates brought together were of opposite compatibility types, fertile perithecia formed within 3 days.

EXPERIMENTAL RESULTS.—Of the 43 naturally infected trees included in this study, 23 yielded only A isolates, 18 yielded only B isolates, and 2 trees gave some A isolates and some B isolates. No cultures were mixtures of A and B.

The results of the artificially inoculated trees are in Table 1. All isolates recovered from the same tree were of the same compatibility type, with the exception of 1 mixed A and B isolate from tree No. 13. Only the B type was recovered from trees No. 1 and No. 6, while the A type was recovered from the other 4 trees. Culture 633 was isolated from trees No. 1 and No. 6 but only once in mixed culture from tree No. 13. Culture 613 was not recovered from either tree No. 2 or No. 3.

Culture 805, one of the components of the mixed conidia inoculum of tree No. 13, was shown by previous study to be a light, unisexual male (2). All the isolates from this tree (except the 1 mixed culture) were likewise light males of the A compatibility type. There were no apparent variations in isolates which might be attributed to passage through the oak tree.

DISCUSSION AND SUMMARY

These results support the previous meager information that few naturally infected oak wilt trees yield isolates of both compatibility types of *E. fagacearum*. Both A and B isolates were recovered from only 2 trees of the 43 naturally infected trees sampled. No isolates from these trees were found to be mixtures of A and B types.

The low frequency of isolates of both types from the same tree might be considered by some workers as evidence of infection by only 1 type of spore. However, this assumption would not necessarily be true, as shown by the rather surprising results in Table 1. The recovery of only 1 compatibility type (with the 1 exception previously noted) from each of 6 trees artificially inoculated with known mixtures of A and B spores indicates that there was a selection and survival of 1 type in each tree. The mechanism responsible for survival is not known, but it is not confined to 1 compatibility type.

The factors leading to the survival of 1 isolate over another seem to act soon after infection. The high frequency of survival of only 1 isolate within the tree would explain why so few trees containing both compatibility types have been found.

The implications of these results seem clear. Mycelial mats produced on a tree containing only 1 compatibility type of the fungus are not known to produce perithecia without first being spermatized by spores of the opposite type. It is logical to assume that these spores come from other mats on nearby trees and that insects are important agents of spermatization. The few trees containing both A and B types are in themselves potential sources of perithecia, provided both types produce mycelial mats in close proximity to each other.

The frequency with which trees bearing mats with perithecia have been found (4, 8) seems to be considerably greater than the frequency with which trees containing both compatibility types have been found. Therefore, these results are interpreted as evidence for the view that in nature perithecia are normally produced following the transfer of conidia between mycelial mats of opposite compatibility types, rather than the intermingling of mycelia in the same tree.

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THE CULTURE OF ERYSIPHE CICHORACEARUM ON SUNFLOWER TUMOR TISSUE [1]

Jean M. Heim and George A. Gries

Preliminary attempts to culture the powdery mildew *Erysiphe cichoracearum* DC. on sterile tumor tissue of sunflower have met with limited success. Since the experiments have been temporarily discontinued this paper presents the methods and techniques used. The goal of culturing an obligate parasite on excised tissue *in vitro* was first realized by Morel[2] who reported the culture of the pathogens causing the downy and powdery mildews of grape on grape callus tissue. More recently Hotson and Cutter[3] and Cutter[4] have cultured rusts *in vitro* on host tissues and have further attained growth of a rust on an artificial medium.

Pure cultures of superficial obligate parasites such as the powdery mildews can not be obtained by the usual techniques of isolating plant pathogens. The method, used by Morel, of inoculating tissue cultures with conidia from nonsterile plants is undesirable because of the high percentage of contamination which occurs. It therefore seemed preferable to produce the inoculum by growing the fungus on sunflower seedlings under sterile conditions. This was done in the following manner.

Seeds of *Helianthus annuus* L. were surface sterilized by immersing them for 15 minutes in a 2.6 per cent solution of sodium hypochlorite. They were then rinsed twice with sterile tap water and placed with sterile forceps on petri plates containing potato-dextrose agar. The radicles started to develop within 1–2 days depending on the incubation temperature. Seeds which germinated normally and appeared to be uncontaminated were transferred aseptically to the sterile containers in which they were to be grown. The culture flasks were prepared by centering 100 ml. beakers in wide-mouthed 2-liter flasks, pouring a molten medium consisting of Hoagland's nutrient solution plus 0.6 per cent agar into the beakers, plugging the flasks with gauze-covered cotton, and autoclaving for 20 minutes at 15 lb. pressure. The beakers served both to support and center the plants and to reduce the amount of medium required. The flasks containing the seedlings were placed in an artificially illuminated room in which the temperature could usually be maintained between 22 and 25°C. Illumination was provided by 12–16 white, 40 watt fluorescent lamps suspended 2¾ feet above the bench on which the flasks were set. A 17-hour day length was used. Growth of plants under these conditions was reasonably normal. At about the time the cotyledons diverged the seedlings were inoculated with conidia from plants in the greenhouse. The conidia were transferred directly to the hypocotyls and cotyledons of the sterile plants with a dry, sterile needle. The inoculated seedlings were replaced in the artificially illuminated room and in 5–10 days mildew appeared on them. The mildew grew well and spread over much of the plant surface. Conidia produced on these plants were then transferred to other sterile plants. After several successive transfers, mildew suitable for the inoculation of the tissue cultures was obtained.

Both tumor and callus tissues of sunflower were maintained *in vitro* for inoculation with *E. cichoracearum*. The tumor tissue used was derived from secondary crown-galls induced on *Helianthus annuus* L. by *Agrobacterium tumefaciens* (Smith and Townsend) Conn. Stock cultures of this tissue were obtained from Dr. A. C. Hildebrandt. The method of maintaining

[1] Accepted for publication March 9, 1953.
Published as Journal Paper No. 687 of the Purdue University Agricultural Experiment Station, Lafayette, Indiana.

[2] Morel, G. 1948. Recherches sur la culture associée de parasites obligatoires et de tissus végétaux. Ann. des Épiph. 14: 1-112.

[3] Hotson, H. H., and V. M. Cutter, Jr. 1951. The isolation and culture of Gymnosporangium juniperi-virginianae Schw. upon artificial media. Proc. Nat. Acad. Sci. 37: 400-403.

[4] Cutter, V. M., Jr. Observations on the growth of Uromyces caladii in tissue cultures of Arisaema triphyllum. Paper presented at joint session of the American Phytopathological Society and the Mycological Society of America, Ithaca, New York, September 8-10, 1952.

these tissue cultures was similar to that of Riker and Gutsche[5] and consisted of growing the tissues in 7-oz. medicine bottles placed on their narrow sides and containing 40 ml. of medium each. The medium used was a sucrose-nutrient agar described by Riker and Gutsche for the culture of sunflower tumor tissue. This tissue was crisp, and was easily cut or broken. It was light cream-colored, and in some cases slightly translucent. The color did not change with the age of the tissue. Growth from some areas on any given transplant was usually more rapid than from others so that the pieces of tissue became irregularly shaped. From 1 to 4 pieces of tissue were grown in each bottle.

The sunflower callus tissue used in these experiments was derived from cultures supplied by Dr. J. H. M. Henderson. The tissue was originally produced from stem sections of 30- to 40-day old sunflower plants. The basal medium on which it was grown was that recommended by Henderson et al[6] and differed from that used for the tumor tissue only in respect to the accessory growth factors (vitamins, adenine sulphate, and coconut milk) and the agar concentration. This tissue grows more rapidly than does the tumor tissue and changes in color from white to cream with age.

Two methods of inoculating the tissues with *E. cichoracearum* were attempted. In one case conidia were transferred from the mildewed plants with a dry sterile needle to the upper surface of the tissue. The other method consisted of placing a strip of epidermis from the infected plant on top of the tissue. This latter method never resulted in infection. Inoculated tissues were incubated in the same artificially illuminated room in which the sterile and mildewed sunflower plants were grown.

[5] Riker, A. J. and Alice E. Gutsche. 1948. The growth of sunflower tissue in vitro on synthetic media with various organic and inorganic sources of nitrogen. Amer. Jour. Bot. 35: 227-238.

[6] Henderson, J. H. M., Mary E. Durrell, and J. Bonner. 1952. The culture of normal sunflower stem callus. Amer. Jour. Bot. 39: 467-473.

About 3 weeks after inoculation, a small white area was observed on 1 piece of tumor tissue. By the end of 4 weeks, white aerial hyphae (conidial chains) could be seen, and their number increased steadily. At this time the temperature in the culture room rose to almost 27° C. and remained there for about 24 hours. Concurrently the aerial hyphae crinkled and, even though the temperature was subsequently lowered, they did not reform. The infected area enlarged little, if any, after this, even though the host tissue continued to grow and appeared healthy except for a small brown spot at the site of the infected area.

In a subsequent group of inoculated tissues, mildew again developed on 1 piece of tumor tissue. The course of development of this mildew colony was much the same as that of the former colony. That is, for a period of time the colony enlarged in area and produced many aerial hyphae after which growth subsided.

Results of these tests demonstrate that the powdery mildew, *E. cichoracearum*, is capable of growth on excised, undifferentiated tumor tissue. Why these 2 pieces of tumor tissue (representing about 2 per cent of the number inoculated) became infected while other pieces inoculated at the same and at different times did not is not known, nor is it apparent why no infection occurred on the callus tissue. Environmental conditions, especially temperature and the amount of moisture on the individual tissue surfaces, are probably influencing factors. It is well known that obligate parasites thrive best on vigorous hosts. This phenomenon conceivably could be carried over to *in vitro* cultures, for it was observed that the pieces of tissue which became infected were growing more rapidly than most of the other pieces of tissue at the time of inoculation.

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PHYTOPATHOLOGICAL NOTES

The Occurrence of a Virulent Race of Phytophthora Infestans on Late Blight Resistant Tomato Stocks[1]. Robert A. Conover and James M. Walter. In a recent report on hereditary resistance to late blight of tomato, Walter and Conover[2] pointed out that "resistance to late blight in green-wrap tomato varieties for a region such as southern Florida may be attained with relative ease only to be lost quickly because of the development of, or invasion by, a different biotype of the pathogen." This note reports such an occurrence and the observations and experimental evidence leading to this conclusion.

The 1951-52 planting of late blight resistant tomato stocks was set in the field on the Sub-Tropical Experiment Station's Highlands Farm, Homestead, Florida, in mid-January. By early February late blight had killed many plants of susceptible parental types; stocks of hybrid origin and resistant parental types were apparently reacting as expected, casual observations revealing that some lines were uniform for resistance while others were segregating. However, on February 20 close examination revealed that resistant parental stocks P–1 and P–3 were showing occasional normal, sporulating lesions in addition to the abortive lesions characteristic of plants resistant to infection

[1] Florida Agricultural Experiment Station Journal Series No. 91.

[2] Walter, J. M. and R. A. Conover. 1952. Hereditary resistance to late blight of tomato. Phytopathology 42: 197-199.

with *Phytophthora infestans* (Mont.) dBy. Normal lesions were also found on plants of lines homozygous for resistance and on the apparently resistant plants of segregating lines. The proportion of normal lesions increased gradually, so that all stocks were so severely damaged by early April that no line would have been rated resistant by standards used previously.

This field reaction suggested that the apparent lack of resistance was due to a virulent race of *P. infestans* not present in previous seasons. Had other factors, such as temperature or physiological condition of the plants, been responsible it is likely that individual plants would have shown a uniform reaction to late blight instead of showing both normal and abortive lesions. Since it seemed desirable to determine whether or not a new race of the pathogen was involved, the following experiment was made.

In order to permit a direct comparison with the 1950–51 field test, the following tomato stocks were grown from the same lots of seed as used for that planting: Mo. S–34, a commercial-type tomato very susceptible to late blight; P–1, a primitive tomato that had displayed uniform resistance for several seasons; 2–3 PAStW, a selection from the F_2 of P–1 × Manahill that showed uniform resistance equal to P–1 in the 1950–51 planting; 6 PAStW, the F_2 of STEP 181 (a Manahill selection) × 2–3 PAStW which was segregating for resistance in the 1950–51 planting. These lines were seeded in flats, each line being located at random and each flat handled as a replicate.

Collections of *P. infestans* were obtained from several naturally infected fruits from the following locations: 1) The planting of late blight resistant stocks at Highlands Farm. 2) A commercial field in Dade County, Florida, 3 miles from the Highlands Farm. 3) A commercial field near Bradenton, Florida.

These fruits were placed in moist chambers and inoculum secured by washing off the zoosporangia with water. Each suspension was atomized on the seedlings in 4 flats (replicates), a total of approximately 80 seedlings of each line thus being inoculated with each collection. After inoculation the flats were placed in a moist room for 24 hours. When inoculated, the rapidly growing seedlings bore from 2–4 true leaves each. Seven days after inoculation the seedlings were examined and each placed in one of the disease index classes proposed by Gallegly [3].

The results clearly showed distinct differences in virulence of the 3 collections of *P. infestans*. P–1 and 2–3 PAStW were uniformly resistant to the Bradenton and Dade County collections while 6 PAStW was segregating and Mo. S–34 was susceptible. These reactions were the same as observed in the field during the 1950–51 season. In contrast, all 4 lines were equally and completely susceptible to the Highands Farm collection. Analysis of the data indicated differences between sources of *P. infestans* and differences between tomato stocks inoculated with the Braden- and Dade County collections to be a significant at odds greatly in excess of 99:1. These results seem to prove conclusively that a virulent race of *P. infestans* was responsible for the susceptibility of resistant tomato stocks in the 1951-52 planting.

Little evidence is available to suggest an origin for the virulent race of *P. infestans*. In previous seasons an increase in susceptibility of resistant plants during senescence has been noted. That this probably does not apply to the present case is indicated by the occurrence of normal lesions on resistant plants prior to fruit set. It does not seem likely that the virulent race originated by the method reported by Mills and Peterson [4] because neither senesence nor the elapsed time appear to fit the conditions they describe for development of new races.

Gallegly [3] has presented evidence to show that there are at least 3 races of *P. infestans* virulent on tomato and that P–1 has resistance to 1 of them. One of Gallegly's races, virulent on P–1, was obtained from Ft. Pierce, Florida. If the new race were introduced from Ft. Pierce, it seems likely it would have been rather widespread in the Homestead area. The collection of a non-virulent race only 3 miles from the virulent collection suggests a hitherto unsuspected localization of races and may indicate a local origin of the virulent race.—University of Florida, Sub-Tropical Experiment Station, Homestead, Florida, and Gulf Coast Experiment Station, Bradenton, Florida.

[3] Gallegly, M. E. 1952. Physiologic races of the tomato late blight fungus. Phytopathology 42: 461-462.

[4] Mills, W. R. and L. C. Peterson. 1952. The development of races of *Phytophthora infestans* (Mont.) de Bary on potato hybrids. (Abs.) Phytopathology 42:26.

Suggested Infection Scales for Roguing Strawberry Seedlings Susceptible to Mycosphaerella fragariae and Diplocarpon earliana.[1] L. P. S. Spangelo[2] and A. T. Bolton.[3] In the strawberry breeding program of the Division of Horticulture, Central Experimental Farm, Ottawa, Ontario, Canada, an attempt has been made to avoid the selection of seedlings which are highly susceptible to *Mycosphaerella fragariae* (Schw.) Lind. (leaf spot) and *Diplocarpon earliana* (Ell. and Ev.) Wolf. (leaf scorch).

Six infection classes for each disease have been photographically defined to facilitate the roguing of susceptible seedlings. The 2 infection scales are presented in Figure 1, A and B. The disease rating of each seedling is based on the most heavily infected leaf and is made during the late fall of the year previous to the first fruiting season.

A typical leaf spot lesion with a greyish-white centre of conidiophores is shown in Figure 2, A. Leaf scorch is identified by the presence of acervuli on the lesions,

[1] Contribution No. 801 from the Division of Horticulture, Experimental Farms Service, and Contribution No. 1228 from the Division of Botany and Plant Pathology, Science Service, Department of Agriculture, Ottawa, Ontario, Canada.

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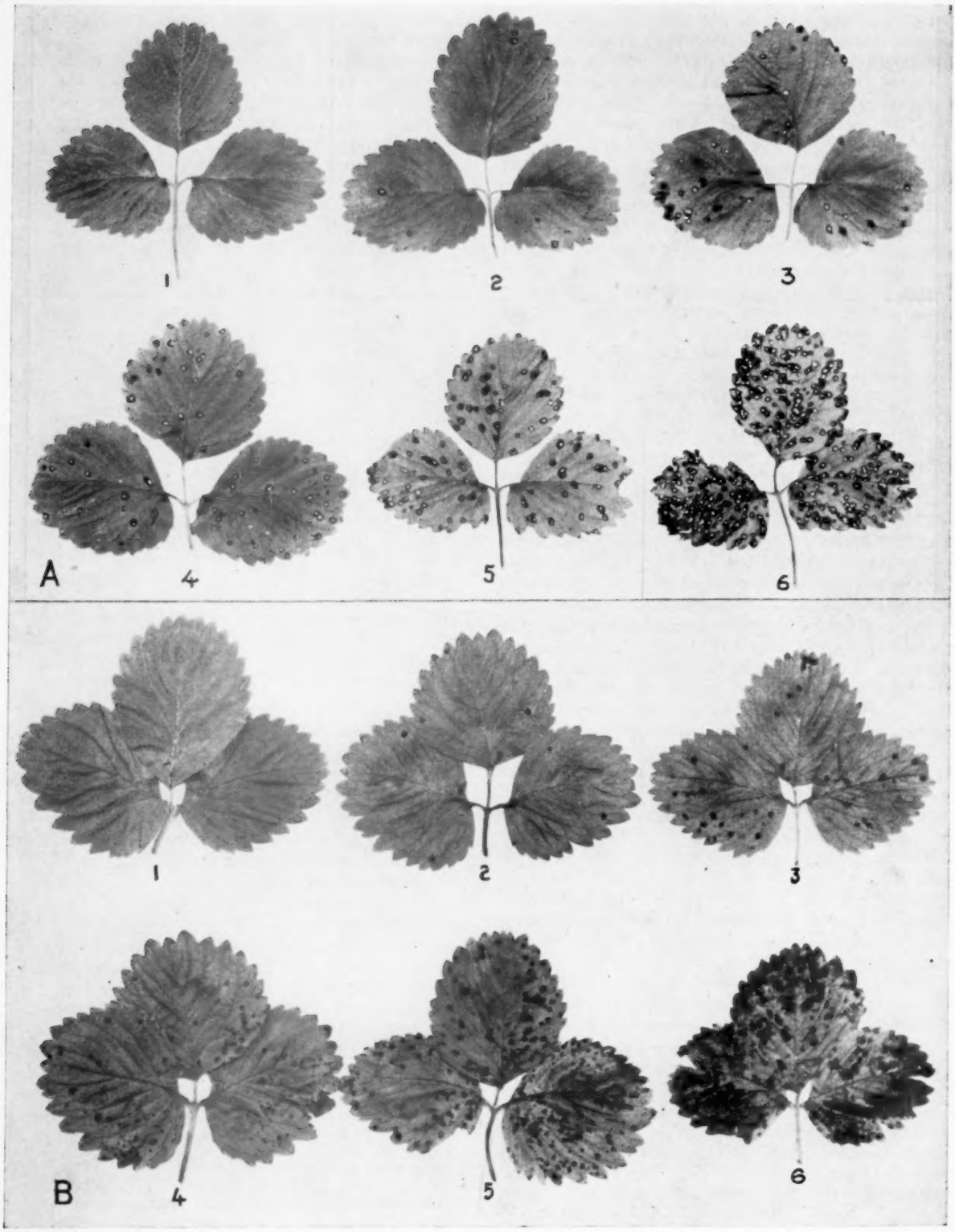

FIG. 1 A) Leaf spot infection scale (Photograph by A. Kellett); B) Leaf scorch infection scale (Photograph by A. Kellett).

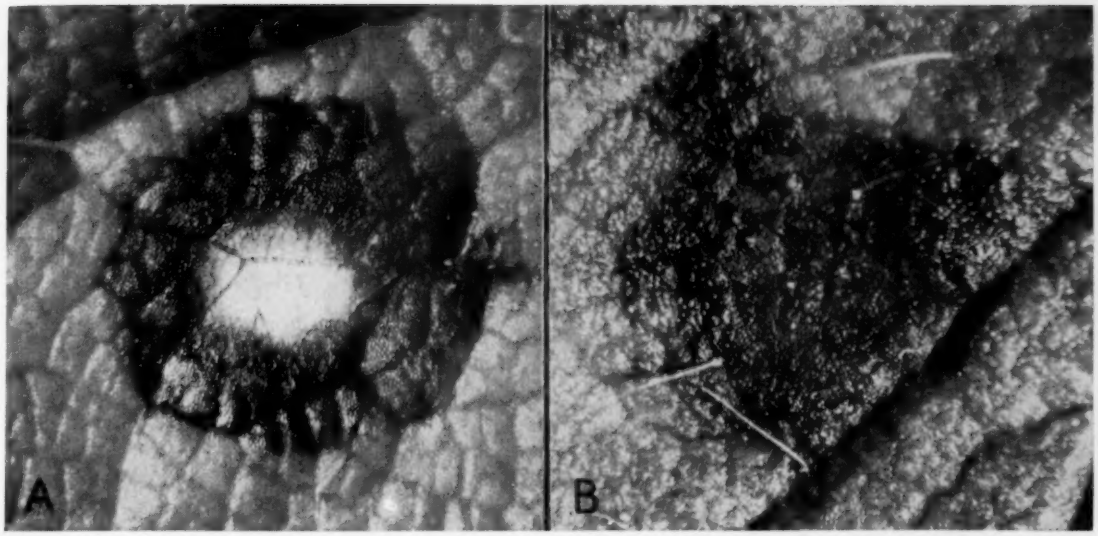

FIG. 2 A) A typical leaf spot lesion with the greyish-white center of conidiophores (24×); B) Acervuli of a typical leaf scorch lesion (24×).

as shown in Figure 2, B. The acervuli are readily seen with a hand lens of 12 magnification.

Highly, moderately, or slightly susceptible, are infection classes commonly used for rating susceptibility to leaf spot and leaf scorch. Unless accompanied by extensive descriptions, data collected in different seasons or by different workers and presented in this form are not readily comparable. It is suggested that clearly defined photographic infection scales, such as those presented in Figure 1, A and B, would standardize disease readings and would make comparison easier.—Central Experimental Farm, Ottawa and Division of Botany and Plant Pathology, Department of Agriculture, Ottawa, Ontario, Canada.

Melanconium Leaf and Stem Fleck of Grapes.[1] E. S. LUTTRELL. Bitter rot, caused by *Melanconium fuligineum* (Scrib. & Viala) Cav., is the most important berry rot of muscadine grapes (*Vitis rotundifolia* Michx.). It occurs on bunch grapes as well, although on this host it is of less importance than black rot and anthracnose. *M. fuligineum* has also been found to cause mild leaf and stem infection of both muscadine and bunch grapes. Infection of the vegetative parts was first observed in muscadine vineyards at Tifton and Experiment, Georgia, in May 1945. It has been observed occasionally during the spring in subsequent years and has been found also on cultivated Warren bunch grapes (*V. bourquina* Munson *ex* Viala) and wild bunch grapes.

Infection of the leaves (Fig. 1) produces tiny, sunken, dark reddish-brown lesions rarely more than 0.1–0.3 mm. in diameter. The lesions are surrounded by narrow, pale yellowish haloes. The lesions are more numerous on the lower surface of the veins and may coalesce to turn segments of the veins brown. On petioles, tendrils, and stems the lesions are round to long-elliptic, slightly raised, dark reddish-brown to black spots and streaks up to 1.5 mm. long. They may be crowded to form interrupted crusts.

Isolations from infected leaf and stem tissue of

TABLE 1.—*Results of inoculations with Melanconium fuligineum on vegetative shoots of grapes*

Host	Source of inoculum: Conidia from muscadine berry — Shoots inoculated	Shoots infected	Leaves inoculated	Leaves infected
	number	per cent	number	per cent
Vitis rotundifolia	7	100	43	58
Vitis bourquina	2	100	13	85
Vitis labrusca	2	100	11	9
	Conidia from culture from muscadine tendril — Shoots inoculated	Shoots infected	Leaves inoculated	Leaves infected
	number	per cent	number	per cent
Vitis rotundifolia	7	85	46	37
Vitis bourquina	5	100	30	63
Vitis labrusca	1	0	7	0
	Control — Shoots	Shoots infected	Leaves	Leaves infected
	number	per cent	number	per cent
Vitis rotundifolia	11	0	77	0
Vitis bourquina	5	0	30	0
Vitis labrusca	3	0	16	0

[1] Paper No. 234, Journal Series, Georgia Experiment Station.

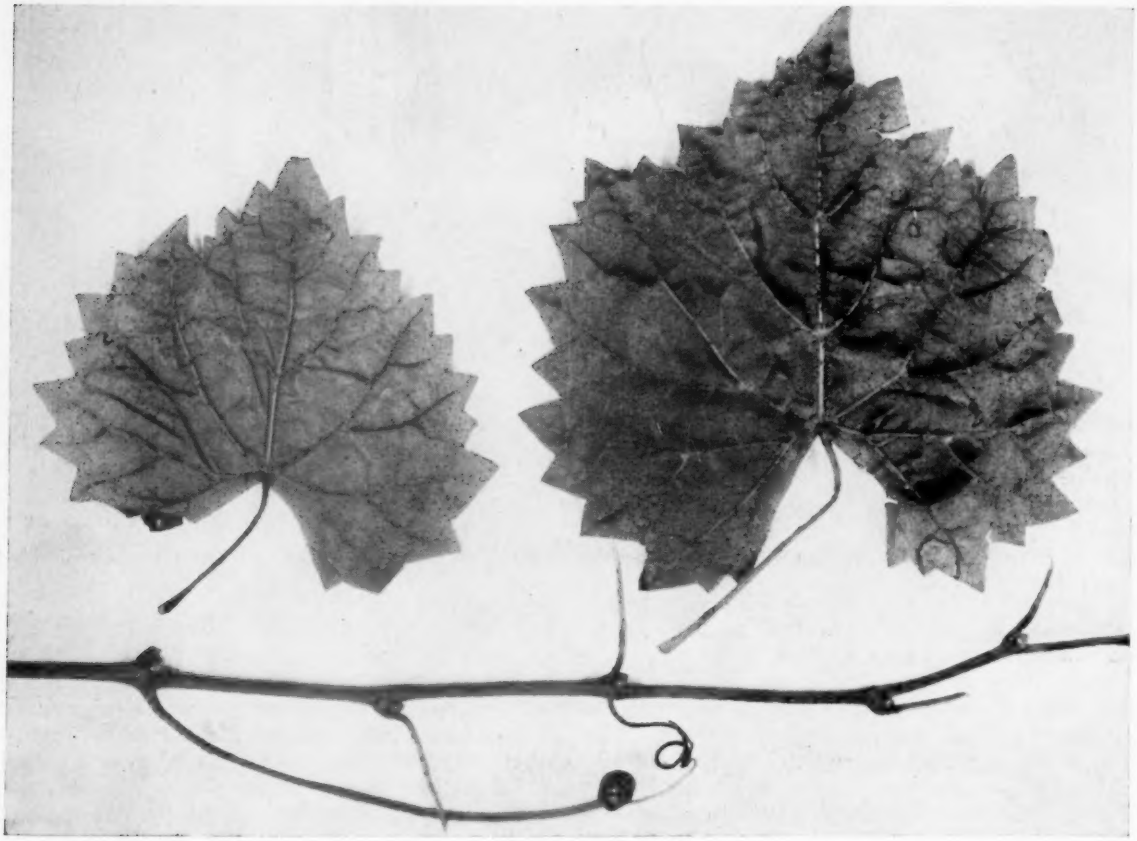

FIG. 1. Leaf and stem fleck of muscadine grape caused by *Melanconium fulgineum*.

muscadine and bunch grapes consistently yielded cultures of *Melanconium fuligineum*. Inoculations were made on young, vigorously-growing shoots of muscadine grape, Warren bunch grape, and Concord bunch grape (*Vitis labrusca* L.) grown in pots in the greenhouse. Shoots were sprayed with a suspension of conidia from infected muscadine berries from the field and a suspension of conidia produced in culture by an isolate from an infected muscadine tendril. The plants were placed in an inoculation chamber for 48 hours. Results are in Table 1. Conidia from both sources produced infection on the young leaves and stems of both muscadine and bunch grapes. Noninoculated shoots, which served as controls, did not become infected. These inoculations indicate that *M. fuligineum* is capable of infecting vegetative parts as well as the berries of grapes and also that the same fungus may infect both muscadine and bunch grapes.

Melanconium leaf and stem fleck is so rare and of such little consequence when present that it is of no practical importance. Since the fungus has not been observed to fruit on leaf and stem lesions, such lesions probably do not serve as a source of later infection on the berries, a function well performed by acervuli on overwintered berry mummies and pedicels.—Georgia Experiment Station, Experiment, Georgia.